Studies of the origins and control of occupational exposure to cytotoxic drugs

Roberts, Sarah

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Studies of the Origins and Control of Occupational Exposure to Cytotoxic Drugs

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Sarah Roberts

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Department of Pharmacy and Pharmacology

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Signature :……. Date:……..

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Abstract

A three-part project was devised to investigate the origins of and potential methods to reduce the risk of occupational exposure to cytotoxic drugs. The first phase involved researching the current decontamination methods applied in UK hospital pharmacies, which manipulate cytotoxic drugs. The second phase evaluated practical decontamination methods, and the third phase investigated one intervention aimed at reducing or preventing contamination occurring in an isolator.

A questionnaire was sent out to ASU managers in NHS hospital pharmacies to gain information about the disinfection and decontamination procedures and products used. The practical decontamination methods investigated were mechanical removal and degradation by detergents (pH range from 1.7 - 13.2) and cleaning agents, and degradation by vaporised hydrogen peroxide. Analytical methods were developed and validated to recover and quantify the amount of cytotoxic marker drug remaining after the decontamination tests carried out in phase two, and to recover and quantify cytotoxic surface contamination from various surfaces in phase three of this work. This composed an attempt to evaluate the effectiveness of a closed-system e.g. PhaSeal® device for fluid-transfer, in reducing contamination produced from the compounding of cytotoxic drugs in an isolator.

The detergents and cleaning agents were effective in removing or reducing cytotoxic surface contamination. Alkaline detergents caused degradation of doxorubicin (maximum 81% at pH 13.2 after 1 hour exposure); the other detergents tested did not

degrade the cytotoxic drugs investigated. Exposure to vaporised hydrogen peroxide (1.6 g min^{-1} for 2 hours) caused the degradation of cyclophosphamide (98.9%), 5-Fluorouracil (29.3%), doxorubicin (71.0%) and epirubicin (65.9%) when exposed in pharmaceutical diluents. The closed-system (PhaSeal®) device was effective in reducing contamination produced in an isolator from the compounding of cytotoxic drugs.

The risk posed by handling and manipulation of cytotoxic drugs and products to the operator and the environment may be reduced, if not eliminated by considering additional approaches to the methods already in place. Firstly, the application of effective decontamination methods; and secondly, by using an effective closed-system, for example the PhaSeal® drug transfer device in a controlled environment.

List of Abbreviations

ASU	Aseptic Services Unit
BSC	Biological Safety Cabinet
CIVAS	Centralised Intravenous Additive Services
CP	Cyclophosphamide
CV	Coefficient of Variation
DOX	Doxorubicin
EPI	Epirubicin
5-FU	5-Fluorouracil
HPLC	High-Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
IMS	Denatured ethanol, 70% v/v
IPA	Isopropyl alcohol, 70% v/v
LoD	Limit of Detection
LoQ	Limit of Quantification
MABs	Monoclonal Antibodies
MTX	Methotrexate
NS	Normal Saline, 0.9%
QACs	Quaternary Ammonium Compounds
QS	Quinine Sulfate
VHP®	Vaporised Hydrogen Peroxide
WFI	Water For Injections

1. Literature Review

1.1 Literature Search Strategy

The references for the following literature review were retrieved as a result of searching databases from the Web of Science, Pubmed, Embase and Science Direct. A variety of search terms were used alone and in combination, to build a database of references for the study.

In general, there were plentiful references in this area of study. Additional information was gathered from various sources other than peer-reviewed journal literature, including textbooks, conference abstracts and posters, technical data files, monographs and the World Wide Web. Personal communications are also referred to, where their inclusion is of importance. All sources are referenced.

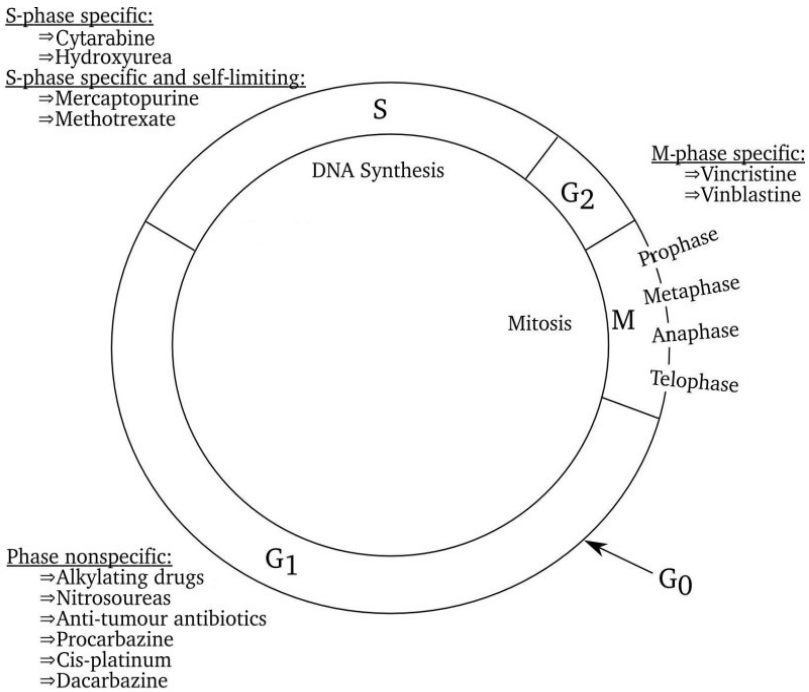
1.2 Cytotoxic Chemotherapy

Cytotoxic drugs are the only systemic treatment for cancer, and are used either as a single modality treatment, or in combination with adjuvant therapy *i.e.* radiation or surgery. The approaches of radiation and surgery often eradicate localised disease but may fail if the cancer has metastasized to other areas of the body. Surgery or radiation, or both combined with chemotherapy, have increased survival rates for a number of solid tumours.[1] The clinical use of cytotoxic drugs is a paramount necessity in the successful treatment of cancer and the number of patients being cured of cancer successfully is increasing gradually every year.[2] These drugs are also effective in the treatment of non-cancer disease. Effective chemotherapy may considerably improve the quality of life, and extend the life of the patient, even when there is a poor chance of survival. Patients receiving chemotherapy have lead almost normal lives due to relief from some of the symptoms of the disease, when previous treatment with

radiation or surgery has been unsuccessful, highlighting the immense benefit of chemotherapy as a palliative therapy.[2] A more recent approach is to combine cytotoxic therapy with monoclonal antibodies (MABs) *e.g.* the addition of trastazumab to anthracyclines for the treatment of breast cancer. Direct administration of MABs has had limited success, but adjuvant chemotherapy with anthracyclines have been demonstrated to be superior with respect to disease-free and overall survival from breast cancer.[3]

Cytotoxic drug use has increased gradually over time due to more agents being synthesised/discovered, the use of ambulatory infusion devices, combination and adjuvant chemotherapy and improved supportive care. However, cytotoxic drugs are not just selective for cancer cells and cytotoxic effects are also directed at the growth and reproduction of healthy cells. Single agents can be effective but it is more common to use drug combinations, increasing the possibility of inflicting cell damage in all stages of the cell cycle (see Figure 1 below).

Figure 1. The Cell Cycle and the Relationship of Cytotoxic Drug Action to the Cycle



The only reason chemotherapy is feasible is that normal tissues may recover more rapidly, and the life of the patient is prolonged. The risk of adverse effects however, is not justifiable to healthy personnel; an estimated number exceeding 5.5 million may be exposed to sub-therapeutic levels of these drugs in their occupational environment *e.g.* pharmacists, medical and nursing staff.[4]

Cytotoxic drugs are classified according to pharmacologic action or effect on cell reproduction and encompass a diverse range of chemical structures:

1.2.1 Covalent DNA-Binding Drugs

The covalent DNA-binding drugs are highly reactive compounds. They act as DNA cross-linkers by binding covalently to nucleophilic groups on cellular constituents. The nucleophilic groups of proteins or nucleic acids *e.g.* amino, carboxyl, sulfhydryl or imidazole moieties are substrates for chemical attack by the covalent DNA-binding drugs. They may also be referred to as alkylating agents, as the nucleophilic group which binds to the DNA is an alkyl group.[1] Bifunctional alkylators are the most reactive as they are able to form more cross-linkages with DNA than monofunctional alkylators. These linkages can occur during any stage of the cell cycle. In general, they attack exocyclic oxygens and ring nitrogens.[1] Some of the most damaging agents are those which methylate the O-6 position of guanine, producing a mutagenic and carcinogenic lesion if not repaired.[2] There are six major groups of covalent DNA-binding drugs, five of these groups are alkylating agents.

The covalent DNA-binding drugs are grouped as follows:

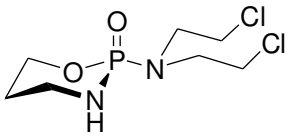
Nitrogen Mustards

The nitrogen mustards are cyclophosphamide (CP), ifosfamide (IFOS), chlorambucil, melphalan and mechlorethamine (nitrogen mustard gas).[1] The proposed

mechanism of action is a multi-step mechanism: at neutral or alkaline pH, the β-carbon of the chlorethyl chain undergoes intramolecular nucleophilic attack by the nitrogen, releasing a chloride ion.[5;6] The aziridinium ion intermediate formed (a strained three-membered aziridine ring) is highly reactive and is subject to intermolecular attack by nucleophiles, causing opening of the ring and alkylation of the nucleophile.[5;6] Typically, a covalent bond is formed between the drug and the N-7 group of guanine.[6] The nitrogen mustards are bifunctional alkylators. They contain two chlorethyl chains and can undergo another cyclization, forming a second covalent bond with another nucleophilic group. The biological effect observed depends on the result of cross linking between strands of DNA or between bases on the same strand of DNA.[1]

The chemical structure of CP is shown in Figure 2 below. Both CP and IFOS (a structural analogue of CP), are oxasophosphorine pro-drugs and require metabolic activation in the liver by cytochrome P-450 mixed function oxidase before they can alkylate cellular constituents.[1]

Figure 2. Chemical Structure of Cyclophosphamide

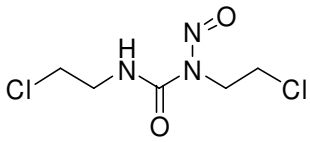


Cyclophosphamide

Nitrosoureas

Carmustine, lomustine, semustine and streptozotocin are the nitrosoureas. They all contain one chlorethyl side chain; except for carmustine, which contains two chlorethyl chains (see Figure 3 below). They are bifunctional alkylators and are also carbamoylators.[1]

Figure 3. Chemical Structure of Carmustine

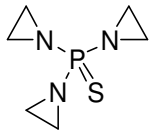


Carmustine

Aziridines

The aziridines are thiotepa (see Figure 4 below) and triethylenemelamine.[1] They both contain a three-membered aziridine ring which is analogous structurally to the aziridinium ion intermediate formed by the nitrogen mustards.[1] Mitomycin is an antibiotic aziridine, which acts as a bifunctional alkylator through a unique mechanism.[1]

Figure 4. Chemical Structure of Thiotepa

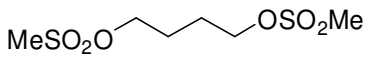


Thiotepa

Alkane Sulfonates

The alkane sulfonates busulfan and myleran are both bifunctional alkylators. Busulfan (see Figure 5 below) reacts with nucleophiles through a rate-limiting reaction depending upon both the concentrations of the drug and the nucleophile.[1]

Figure 5. Chemical Structure of Busulfan

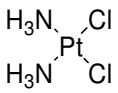


Busulfan

Platinum Compounds

The platinum compounds include oxaliplatin, carboplatin and its analogue cisplatin (see Figure 6 below). They act as DNA cross-linkers, forming strong covalent bonds to produce platinum abducts. Antineoplastic activity results from an interaction with DNA, forming both intra-strand and inter-strand cross-links by the displacement of nucleophilic atoms, particularly the highly reactive 7-nitrogen atoms of guanine and adenine.[1] Cross-links between adjacent guanines on the same strand of DNA are the most readily demonstrated.[1]

Figure 6. Chemical Structure of Cisplatin

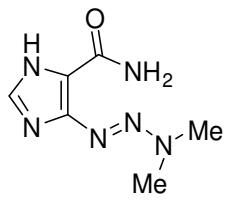


Cisplatin

Methylating Agents

The methylating agents are dacarbazine and procarbazine. These drugs cross-link DNA by methylation, not by alkylation. Dacarbazine (see Figure 7 below) is a prodrug which undergoes cytochrome P-450-mediated oxidative N-demethylation in the liver before becoming an active species.[1] After elimination of the methyldiazonium cation, 5-aminioimidazole-4-carboximide is the active methylating agent, which favours 7-guanine.[1]

Figure 7. Chemical Structure of Dacarbazine



Dacarbazine

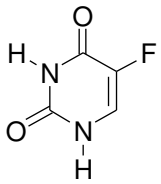
1.2.2 Antimetabolites

The antimetabolites structurally resemble natural metabolites necessary for cellular function. They inhibit *de novo* synthesis either directly by substitution as structural analogues of nucleotides and direct incorporation into nucleic acids, or indirectly by acting as substrates for cellular enzymes inhibiting nucleoside triphosphate production. In both cases DNA and RNA synthesis is inhibited.[1]

Pyrimidine Analogues

The pyrimidine analogues are 5-Fluorouracil (5-FU), fluorodeoxyuridine, CB3717 and azacytidine. They act as direct inhibitors of the enzyme thymidylate synthase (TS).[1] 5-FU (see Figure 8 below) is a fluorine-substituted analogue of uracil and clinically is a very important member of this group. Within the cell, 5-FU is phosphorylated to a nucleoside derivative (5-fluoro-2'-deoxyuridine 5'-monophosphate or 5dUMP) that competes with the natural deoxyribotide of uracil (deoxyuridine 5'-monophosphate or dUMP) for TS. Normally, TS methylates dUMP which is used for the synthesis of thymidine and cytosine in a multi-step process. When 5dUMP is the analogue substrate for TS, the catalysis process becomes frozen at an intermediate step, 5-FdUMP cannot be methylated, and the enzyme, the analogue substrate and the 5,10-methylene tetrahydrofolate cofactor become 'locked' in a stable covalent ternary complex. The trapped enzyme is then inhibited from catalysing thymidine monophosphate synthesis.[1;7]

Figure 8. Chemical Structure of 5-Fluorouracil



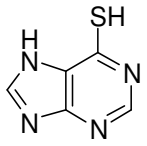
5-Fluorouracil (5-FU)

Purine Analogues

The purine analogues are mercaptopurine (6-MP), thioguanine (6-TG), tiazofurin, chlorodeoxyadenosine and pentostatin.[1] 6-MP and 6-TG are analogues of

hypoxanthine and guanine, respectively.[1] 6-MP (see Figure 9 below) inhibits multiple sites in *de novo* purine synthesis and interferes in the purine salvage pathway by substituting for hypoxanthine as a substrate for hypoxanthine-guanine phosphoribosyl transferase.[7]

Figure 9. Chemical Structure of Mercaptopurine

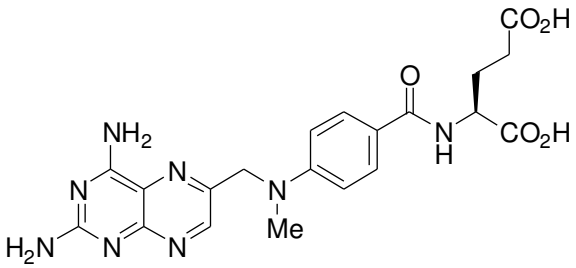


Mercaptopurine

Folate Analogues

The folate analogues are methotrexate (MTX), aminopterin and trimetrexate.[1;7] They are analogues of dihydrofolate and competitively inhibit the action of dihydrofolate reductase, the enzyme responsible for the conversion of dihydrofolate to tetrahydrofolate in the presence of NADPH.[7] Tetrahydrofolate is further converted *via* TS to a coenzyme involved in one carbon transfers to form deoxyuridylate from deoxythymidylate, which is needed in abundance by rapidly dividing cells for the synthesis of DNA.[7] MTX blocks the regeneration of tetrahydrofolate, preventing the synthesis of deoxythymidylate and ultimately halting DNA production. MTX (see Figure 10 on the following page) kills proliferating cells whether they are malignant or not, and as a result can cause significant toxic side effects.[8]

Figure 10. Chemical Structure of Methotrexate

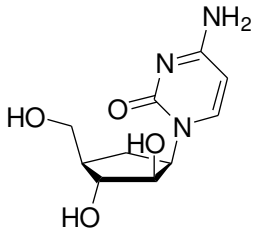


Methotrexate

Sugar-Modified Analogues

Sugar-modified analogues have an altered sugar moiety, rather than a base portion, and are derived from nucleoside analogues.[1] Cytarabine, a nucleoside analogue from a Caribbean sponge[1] (see Figure 11 below), and fludarabine are both sugar-modified analogues.

Figure 11. Chemical Structure of Cytarabine



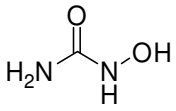
Cytarabine

Ribonucleotide Reductase Inhibitors

Ribonucleotide reductase converts ribonucleoside diphosphates to deoxyribonucleotide diphosphates, and is the only enzyme whose activity is involved in the *de novo* synthesis of all the precursors required for DNA synthesis.[1] This

enzyme is comprised of two protein subunits, one that contains a tyrosyl radical essential for the catalytic activity of the enzyme. Hydroxyurea (see Figure 12 below) is a ribonucleotide reductase inhibitor. Its action is to destabilise the iron centre needed to generate and stabilise the tyrosyl radical, thereby inactivating the enzyme.[1]

Figure 12. Chemical Structure of Hydroxyurea



Hydroxyurea

1.2.3 Non-Covalent DNA-Binding Drugs

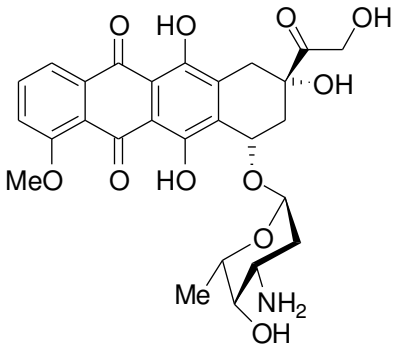
Non-covalent DNA-binding drugs bind to double stranded DNA forming a tight drug-DNA interaction. They all have characteristic planar regions that stack in-between (intercalate) the base pairs, causing them to separate and the double helix to uncoil. The intercalators in routine clinical use are the anti-tumour antibiotics, the anthracenediones, the dactinomycins, bleomycin and pliamycin.[1]

Anti-Tumour Antibiotics (anthracyclines)

Doxorubicin (DOX), daunorubicin, idarubicin and epirubicin (EPI) are anthracycline anti-tumour antibiotics, which are isolated from different species of *Streptomyces*.[1] They consist of a characteristic four-ring structure linked *via* a glycosidic bond to an amino sugar. EPI is an isomer of DOX. Daunorubicin and DOX are identical structurally, except for the presence of a hydroxyl group or hydrogen at

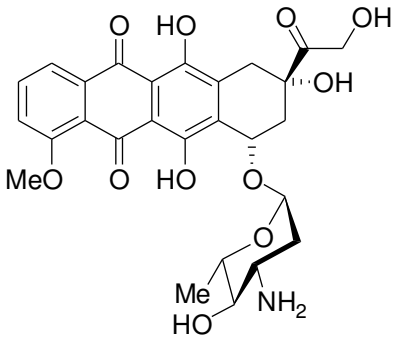
the 14 position of the anthracycline ring.[1] See Figures 13 and 14 below for the chemical structure of DOX and EPI, respectively. The anthracycline antibiotics also interfere with DNA strand breakage and the reunion reaction of topoisomerase II.[1] Furthermore, they can be reduced to a semi-quinone form by NADP and NADPH, generating free radicals that can attack cell membranes and DNA, halting cell growth.[1;8]

Figure 13. Chemical Structure of Doxorubicin



Doxorubicin

Figure 14. Chemical Structure of Epirubicin

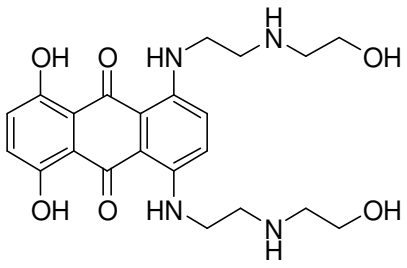


Epirubicin

Anthracenediones

The anthracenediones lack of the sugar moiety of the anthracyclines, but retain the planar polycyclic aromatic ring structure that permits intercalation into DNA. Mitoxantrone (see Figure 15 below) is an example of an anthracenedione;

Figure 15. Chemical Structure of Mitoxantrone

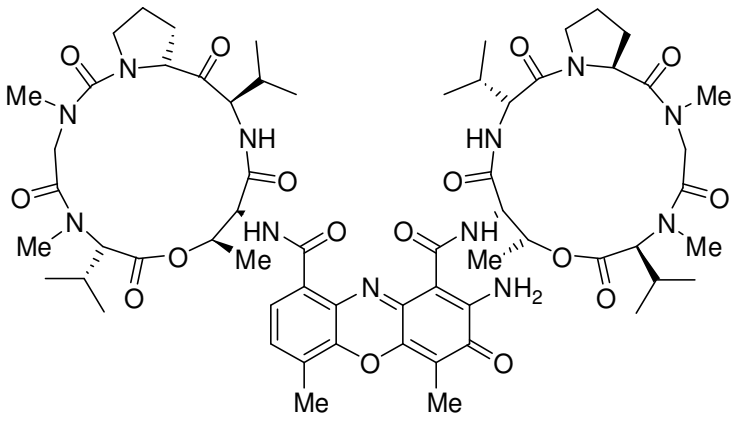


Mitoxantrone

Dactinomycin

Dactinomycin (see Figure 16 below) is an antibiotic from the actinomycin family. Amongst this family dactinomycin is the only drug which is used clinically.[1]

Figure 16. Chemical Structure of Dactinomycin



Dactinomycin

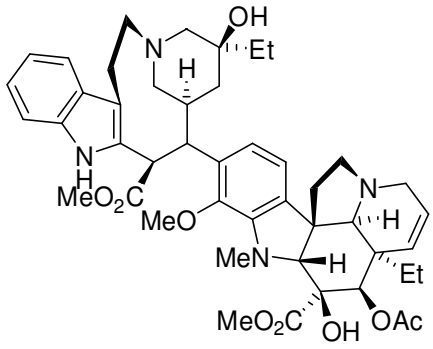
1.2.4 Inhibitors of Chromatin Function

There are two main groups of chromatin inhibitors, the microtubule inhibitors and the topoisomerase inhibitors. The anti-tumour effects of these drugs are thought to be due to the disruption of chromosomal dynamics.[1]

Microtubule Inhibitors

The microtubule inhibitors are the vinca alkaloids *i.e.* vinblastine (see Figure 17 below), vincristine, (derived from the periwinkle plant), vindesine and vinorelbine, and the taxenes *i.e.* paclitaxel (sourced from the bark of the Pacific yew tree), and docetaxel.[1]

Figure 17. Chemical Structure of Vinblastine

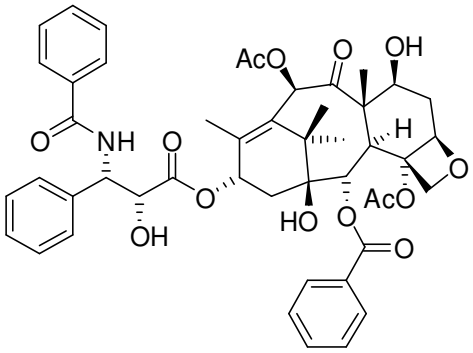


Vinblastine

Microtubules are polymers comprised mainly of the protein tubulin and are responsible for cellular morphology and movement. The vinca alkaloids bind to tubulin and disrupt the balance between microtubule polymerization and depolymerization. This results in dissolution of the microtubules, destruction of the mitotic spindle and arrest of cells, ultimately inhibiting mitosis in metaphase.[1]

Paclitaxel (see Figure 18 below) disrupts the equilibrium between free tubulin and microtubles by shifting the equilibrium towards polymerization, forming abnormal bundles of microtubules.[1]

Figure 18. Chemical Structure of Paclitaxel



Paclitaxel

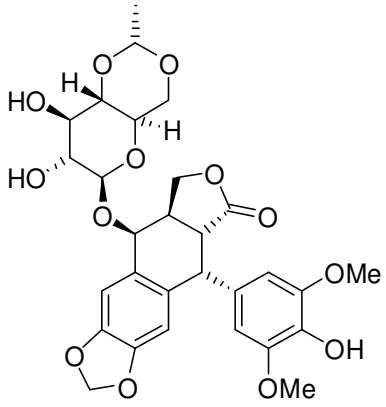
Topoisomerase Inhibitors

Topoisomerases are enzymes that are required to break and reseal strands of DNA, permitting selected regions of DNA to become unwound and relaxed to allow transcription and replication. There are two types of topoisomerases: topoisomerase I, which breaks one strand of DNA, and topoisomerase II, which breaks two strands of DNA. The inhibitors of topoisomerases I are amsacrine and camptothecin, and the inhibitors of topoisomerase II are the epipodophyllotoxins *i.e.* teniposide and etoposide.[1]

The enzyme topoisomerase II interacts with DNA, forming a non-covalent complex. It then cuts both strands of DNA and forms a covalently bound complex between each protein subunit and the 5'-phosphate end of the DNA. Inhibitors of

topoisomerase II, such as etoposide, stabilise this complex and prevent it from returning to the non-covalent complex. In the absence of enzyme inhibitors the complex may be subject to topological changes by strand passage or rotation, the breaks are resealed and the enzyme dissociates from the DNA.[1] See Figure 19 below for the chemical structure of etoposide.

Figure 19. Chemical Structure of Etoposide



Etoposide

As shown in Figure 1 (page 2), some cytotoxic drugs are phase-specific *i.e.* the antimetabolites and inhibitors of chromatin function, and cytotoxic effects are exerted within a specific phase of the cell cycle. Cytarabine and hydroxyurea are S-phase specific, MTX and mercaptopurine are S-phase specific (self-limiting), and vincristine and vinblastine are M-phase specific. The alkylating agents and anti-tumour antibiotics are cell cycle-phase non-specific and can kill cells in any phase of the cell cycle. Even so, they are usually more effective against proliferating cells and may show enhanced activity in a specific phase of the cycle.[1;2]

1.3 Health Effects/Occupational Exposure Risk

The International Agency for Research on Cancer (IARC) monographs represent the most authoritative and scientific series of chemical carcinogenesis information. The purpose of the monographs is to evaluate the carcinogenicity of agents, or environmental exposures, by evaluating the strength of published scientific data. The information provided by the monographs is used internationally to provide a scientific basis for cancer prevention. According to the IARC, at least nine alkylating agents have been classified as carcinogenic to humans (Group1); several have been classified as probably carcinogenic to humans (Group 2A *i.e.* the anti-tumour antibiotics) and possibly carcinogenic to humans (Group 2B). A few (the antimetabolites and mitotic inhibitors) have been classified as not being carcinogenic to humans (Group 3) but are mutagenic and teratogenic.[8;9]

A risk of exposure of healthcare workers to cytotoxic drugs was first reported in the 1970s, when urine samples taken from nurses tested positive for mutagenic substances compared with non-exposed workers.[10] Since then, increasing knowledge of cytotoxic drug toxicity has resulted in growing concern about the potential of cytotoxic drugs to produce adverse effects in healthcare staff handling these agents.

The acute health effects that have been associated with exposure to cytotoxic drugs include direct effects on the mucous membranes, eyes and skin.[11] Symptoms of rashes, dizziness, nausea, vomiting,[11;12] shortness of breath and hair loss have been reported and contact with skin cuts or needle-stick injuries can lead to severe soft tissue injury.[11]

An increase in the number of working mothers in recent decades has resulted in an affect on reproductive health and it is recommended that pregnant women do not work directly with cytotoxic drugs. However, pregnancy is often not acknowledged

until after the first trimester. Within the first 3-month period of the pregnancy, the handling of cytotoxic drugs may still be routine, posing a potential risk to the unborn child. There are 45 cytotoxic drugs listed as Pregnancy Category D by the Food and Drug Administration.[13] Category D includes drugs where there is evidence of risk to the human foetus, but in the therapeutic setting, benefits may outweigh the risk for pregnant women who have a serious condition that cannot be treated effectively with a safer drug. Five drugs are listed as Category X where there is clear evidence that the medication may cause abnormalities to the foetus, but the risks outweigh any potential benefits for women who are or may become pregnant.[13] MTX is one of the five drugs classified as Category X by the FDA.[13] In a study of the effects of the handling of cytotoxic drugs by health workers, menstrual cycle changes among nurses have been reported.[14] Further studies have suggested that women exposed to cytotoxic drugs during or shortly prior to pregnancy are at an increased risk of miscarriage,[15;16] premature delivery and low birth rate.[17] However, no effects on pregnancy outcome have been reported.[18]

Evidence of the effects on the health of staff handling cytotoxic drugs is conflicting; the studies reported vary in design, the level of exposure and the scale of measurement. Many of the studies were conducted in the 1970s prior to the implementation of safety standards, and do not reflect current working practice. To standardise the results, a meta-analysis was conducted that combined and reviewed 14 studies carried out between 1996 and 2004 in the U.S and in Europe. Of these 14 studies, the 7 that were eligible for pooling concluded that insufficient evidence was available to estimate the risk of cancer or acute cytotoxic effects. No significant association between exposure and stillbirths or congenital malformations were measured, but a small increased risk of spontaneous abortion was identified.[19] This

review highlights the lack of data for statistical pooling, and the need for more recent health studies carried out to evaluate the latest safety measures. Studies are required that assess the health effects among humans from exposure to non-therapeutic, environmental levels.

The chronic effects due to long-term repeated exposure to small quantities of these drugs are more concerning, and the actual risks from skin contact, inhalation or dermal penetration are not fully known.[20] There are no data correlating the number of declared symptoms with, for example the frequency of exposures, the drugs exposed to, or the extent of personal protection adopted. Although various adverse health effects have been documented,[18;19] the effect of regular exposure to several drugs over long periods is difficult to quantify.

The effects of long-term exposure can only be assessed over time, thus the results of most studies are based on the occurrence of secondary cancers from chronic exposure. Many cytotoxic drugs, and some drug combinations, have been associated with the development of secondary malignancies, most commonly leukaemia and bladder cancer in patients undergoing potentially curative chemotherapy.[21] In terms of occupational exposure, a significant increase in the incidence of leukaemia among oncology nurses has been reported.[18] As a result of limited available exposure data to assess the cancer risk, animal data, and primary and secondary tumour occurrence in patients receiving CP was modelled.[22] From the available dose-response data and excretory amounts of the drug in urine, a theoretical increase in the risk of leukaemia and bladder cancer exceeding the 'target risk' (no more than one extra cancer case per million per year for one compound) from exposure to this one agent was calculated.[22]

1.4 Cytotoxic Reconstitution Services

Changes have occurred in the last few decades concerning the way cytotoxic drugs are prepared in hospital pharmacies. Cytotoxic reconstitution services allow for the preparation of drugs, under controlled conditions, by appropriately trained staff. These services not only improve occupational safety but also patient safety.[23] The type of cytotoxic reconstitution service is defined locally. The service may be situated close to hospital wards involved in cancer treatments as a satellite to the main hospital pharmacy, or it may be centralised in the main hospital pharmacy, or cytotoxic drugs may be supplied by a single commercial unit to several hospitals within an area.[24]

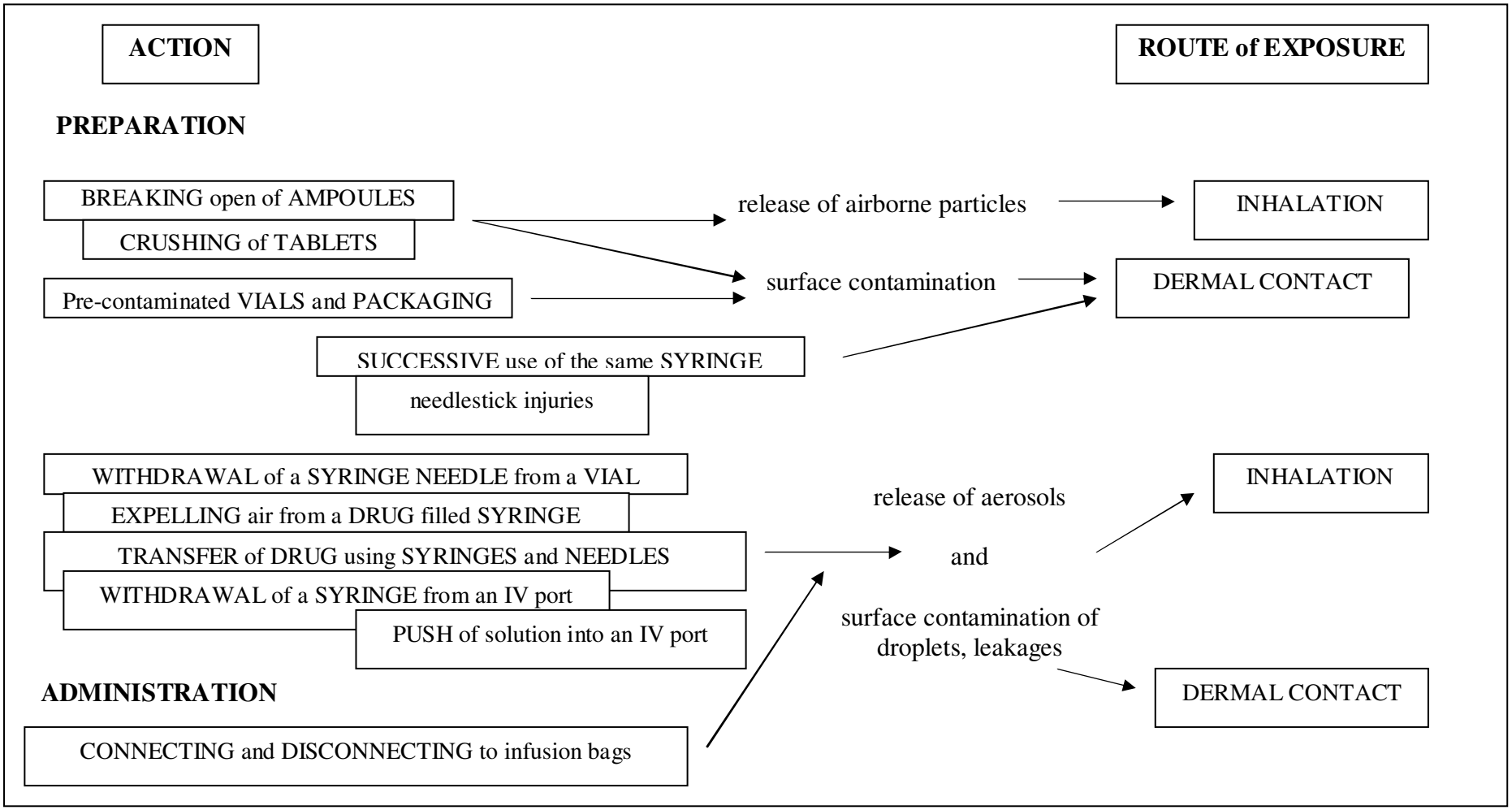
Many European countries have made significant moves towards specially designed centralised units for cytotoxic drug preparation, usually Centralised Intravenous Aseptic Services (CIVAS) within the pharmacy. Drug reconstitution in these facilities, is an aseptic pharmaceutical procedure that is carried out under the direct control of a pharmacist, away from the patient. This leads to a minimal amount of activity on the ward, a reduction of medication errors and centralisation of the risk to occupational exposure in the hospital.[23] In the UK, the Medicines and Healthcare products Regulatory Agency (MHRA) regulate this practice, and health and safety aspects are regulated by the Health and Safety Executive (HSE). In these facilities, pharmacists are less likely to be trained in cytotoxic drug preparation *i.e.* pharmacy technicians and assistants are usually trained 'in-house' to manipulate cytotoxic drugs. Issues concerning the safe handling of cytotoxic drugs are therefore of particular importance to these personnel.

1.5 Routes of Exposure

Despite the implementation of collective protective measures, many international studies have demonstrated evidence of exposure to workers.[25-31] To

reduce the risk it is necessary to consider all possible sources of the exposure. Workplace contamination and subsequent worker exposure may result from several sources during cytotoxic drug preparation and administration. The sources and routes of exposure resulting from cytotoxic drug preparation and administration are shown schematically in Figure 20 on the following page.

Figure 20. Schematic Diagram Showing the Routes of Exposure from the Preparation and Administration of Cytotoxic Drugs



Exposure through inhalation and skin absorption are the primary sources,[4] and are likely to occur from actions taken during the preparation process and from pre-contaminated sources such as the outside of drug vials.

1.5.1 The Preparation Process and Routes of Contamination

Cytotoxic doses can be harmful to the patient if they are not prepared correctly. Prior to administration to the patient, cytotoxic dose preparation involves reconstitution, as many are supplied as lyophilized powders because they are unstable in solution, volume adjustment and dilution.

Inhalation has been attributed to the airborne contamination of drug particles or droplets.[8] The employment of fluorescent markers has been used to identify routes of contamination, as the majority of cytotoxic drugs are colourless and odourless and are not visible to the naked eye. Spiking a solution with a dye is a useful tool to demonstrate drug leakage into the environment during simulations of preparation and administration.[32] Using this method, the production of aerosols was observed from the aseptic transfer of a dye solution to occur in the following ways; by withdrawing a needle from an over-pressurized drug vial; the withdrawal of a needle from the port of an IV bag; the simulated drug administration and the push of the solution into an IV port; transferring drugs using syringes and needles; crushing tablets; breaking open ampoules; expelling air from a drug-filled syringe and connecting and disconnecting to infusions.[32;33]

Drug particles may also become airborne after contamination on surfaces *e.g.* surfaces inside the isolator or biological safety cabinet (BSCs), gloves, vials or trays has dried. It has been reported that carmustine, IFOS, thiotepa and CP may vaporise from surfaces at room temperature, and the vapour pressure of CP is three times higher at 40ºC compared to 20ºC.[34] Certainly, temperatures higher than room

temperature may be reached under normal working conditions, where cytotoxic drugs are manipulated. This may be due to the continual running of isolators and BSCs, or the use of ancillary equipment *e.g.* lighting and the running of pump motors. Particle size also influences the rate of vaporisation and small drug particles may vaporise within seconds or minutes.[34] Aerosols or drug particles may settle on immediate surfaces in the environment and be picked up *i.e.* on gowns, gloves or shoes and transported to other areas, contributing towards surface contamination.

Inhalation alone cannot explain the levels in urine found in operators and evidence suggests that dermal contact and consequently skin absorption may also be a major route of exposure.[20;30;35-37] Dermal exposure may occur through glove penetration, where direct contact with contaminated material may transfer contamination onto gloves.[38] Successive use of the same syringe has also been implicated as a source of contamination. CP was shown to infiltrate onto the plunger of syringes, not after first usage but after several and increasing plunges of the syringe.[39] This would contribute towards glove contamination and hence dermal contact, even in the absence of any manipulation errors.

Oral ingestion or accidental dermal exposure from needle-stick injuries, spillages or breakages are also potential routes of exposure.[4] These events happen less frequently but contribute towards airborne contamination and long-term contamination of the workplace.

1.5.2 Pre-Contaminated Sources

Pre-contaminated sources may also contribute towards glove contamination and consequent dermal contact. It has been demonstrated that the external surface of drug vials are contaminated with the corresponding drug.[40-42] In a study measuring 5-FU contamination on vials available commercially in Belgium, 27 out of 90 vials

were found to be contaminated with traces of 5-FU.[40] The levels found varied according to origin. Three of these vials were contaminated with quantifiable amounts of 5-FU, one as high as 18.1 ug mL^{-1}.[40] External contamination of the corresponding drug ranging from 0.5 to 24 µg per vial was also recovered on all vials filled with 5-FU, etoposide, CP, IFOS, DOX and docetaxel.[41] Quantities varied between drug batches supplied by the same manufacture, and between manufacturers.[41] Carboplatin, cisplatin and MTX contamination have also been reported on the corresponding vial, and vials of CP have been reported to be cross-contaminated with IFOS.[43] It is feasible that drug contamination on vials could also contaminate any corresponding external packaging. Certainly, 5-FU contamination has been recovered from the corresponding vial packaging, and as the vials themselves were also contaminated with 5-FU, they were implicated as the source.[41]

A pre-contaminated source taken into an environment prior to any manipulations being carried out will contaminate gloves, and any surfaces the vials are placed or stored on. This may include surfaces in the preparation area such as bench tops, shelves, trays or the base of the isolator or BSC. The manufacturers' of the drug have control over this source of contamination. Contamination during manufacture may occur during the vial filling process where splashing, foaming or dusting can occur. Accidental breakage of vials during freeze-drying, collisions on conveyor belts or turntables, and accidents during transportation can also happen.[44] Variations in the levels of contamination found on vials from different manufacturers indicate that any precautionary measures taken are not standardised.[40;41] A study has shown that contamination can be reduced after the filling process, by cleaning the vials with a vial washer, and subsequent application of a protective sleeve. This was demonstrated on cisplatin vials, which were washed using a powerful stream of water,

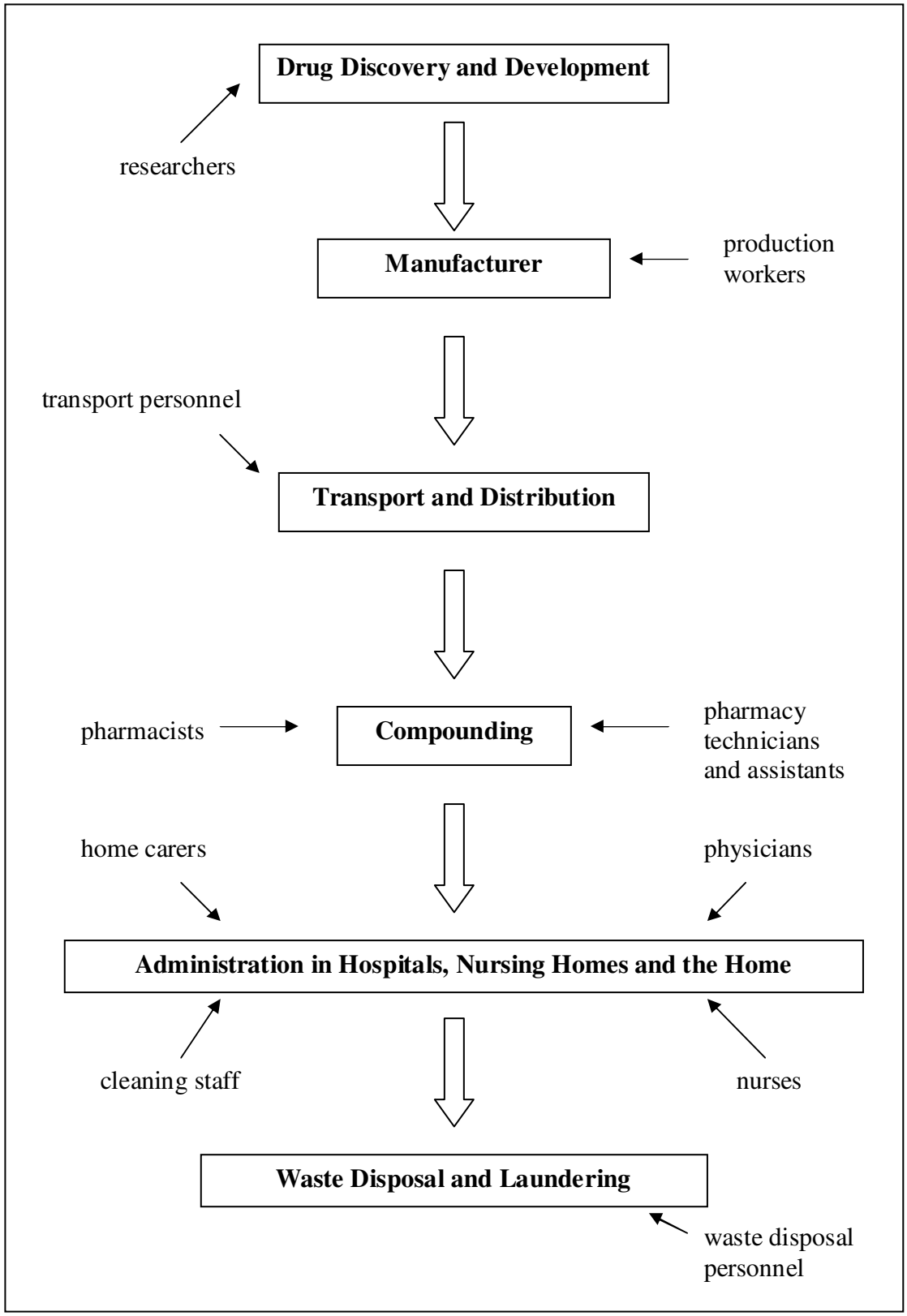
which also decontaminates the base of the vial. Sleeves were then shrunk tightly around the vials to contain any remaining contamination and to serve as a form of protection. The effectiveness of this procedure was demonstrated when levels of platinum contamination on vials of the corresponding drug were reduced by 90%.[45]

1.6 Control Measures/Risk Management/Safe Handling

There appears to be little monitoring data in the UK on potential cytotoxic exposure to staff, albeit the considerable routine quality control test data for product sterility and patient protection.[26] The handling of cytotoxic drugs occurs in a range of surroundings throughout the life cycle of the drug. It is not just restricted to the hospital pharmacy but extends to other populations through a complex chain; from production to transport and distribution; preparation and administration in hospital; nursing homes and home care environments; laundering; disposal and waste treatment. This involves many workers - production workers, transport personnel, pharmacists, pharmacy technicians and assistants, nurses, physicians, home carers, waste disposal personnel, transport staff and cleaning staff. It can also be extended to vets and researchers.[46] These populations are potentially at risk from exposure to these drugs contributing a risk of toxicity to healthy cells.

The interaction of various personnel with cytotoxic drugs from drug discovery through to waste disposal is illustrated in Figure 21 on the following page.

Figure 21. Life Stages of the Cytotoxic Drug and Identification of Personnel at Risk during the Various Stages of Drug Manufacture, Transportation, Storage, Compounding and Administration



Many countries have introduced statutory controls to protect the worker from hazards in the workplace:

- COSHH (Control of Substances Hazardous to Health) UK;

- HSE (Health and Safety Executive) UK:

- OSHA (Occupational Health and Safety Administration) USA;

- NIOSH (National Institute for Occupational Health and Safety) USA;
  (an arm of the better-known OSHA);

Non-statutory guidelines have been drawn up to provide recommendations on good practice:

- OSHA Work Practice Guidelines 1986,[47] 1995[48] and OSHA Technical Manual;[49]

- ASHP (American Society of Health-System Pharmacists) Handling Cytotoxic Drugs in Hospitals;[50] and Technical Assistance Bulletins on Handling of Cytotoxic and Hazardous Drugs;[51]

- ISOPP (International Society of Oncology Pharmacy Practitioners) Standards of Practice. Safe Handling of Cytotoxics;[52]

- HSE Personal Protective Equipment at Work Regulations 1992[53] and HSE information sheets;[54;55]

- SHPA (Society of Hospital Pharmacists of Australia) Standards of Practice for the Safe Handling of Cytotoxic Drugs in Pharmacy Departments;[56]

- RCN (Royal College of Nursing) clinical practice guidelines (1998); the administration of cytotoxic chemotherapy;

- The MARCH (Management and Awareness of the Risks of Cytotoxic Handling) Guidelines.[57]

The MARCH guidelines were developed in acknowledgement of an absence of guidelines specific to chemotherapy pharmacy practice. They are in the form of an internet on-line service and cover all aspects of pharmacy cytotoxic services.

Although the specific recommendations of the guidelines vary between countries, the general principles are the same; controlled handling procedures, a high level of staff training and, if possible, CIVAS with working areas specially designed for cytotoxic containment.

Challenging the traditional assumptions about the safe handling of cytotoxic drugs is universal. Despite the implementation of collective protective measures many international studies have still demonstrated cytotoxic contamination in the surrounding environment,[26;43;58-68] and in the urine of operators.[25-31;69] So great is the concern in the U.S that NIOSH issued an alert in March 2005, to educate healthcare workers of the risk, and to provide guidance on implementing the best measures possible to protect their health.[4]

There are no acceptable safe levels of exposure to cytotoxic drugs, therefore all the necessary measures possible should be employed to keep the levels to a minimum. The effects on health are specific to the drug, the route and amount of exposure and individual physiological variation. Published guidelines (as mentioned previously) have contributed greatly to increased awareness and have provided advice on the recognised risks at the time. One recent study reported a decrease in CP, although still detectable, in the urine of nurses since the introduction of guidelines in 1997.[69] Although many advances have been made, new improved methods to monitor employee risk are needed. Practical steps to prevent harm to the employee should be implemented and should include: identification of the hazards; procedures for emergency spillages including documentation of events; periodic evaluation and

updates of work practices to ensure safe procedures are being followed and that staff are regularly updated; written safe policies and procedures; and the provision of information and training in the occupational health aspect of manipulating and disposing of these drugs.[11]

1.6.1 Engineering Controls (Biological Safety Cabinets and Isolators)

There are two different workstation containment technologies used for the compounding of cytotoxic drugs. The design of these workstations is to contain hazardous materials as well as maintaining a high quality aseptic work area to protect the product. Isolators and BSCs are the main choice of use for the aseptic manipulation of cytotoxic drugs.

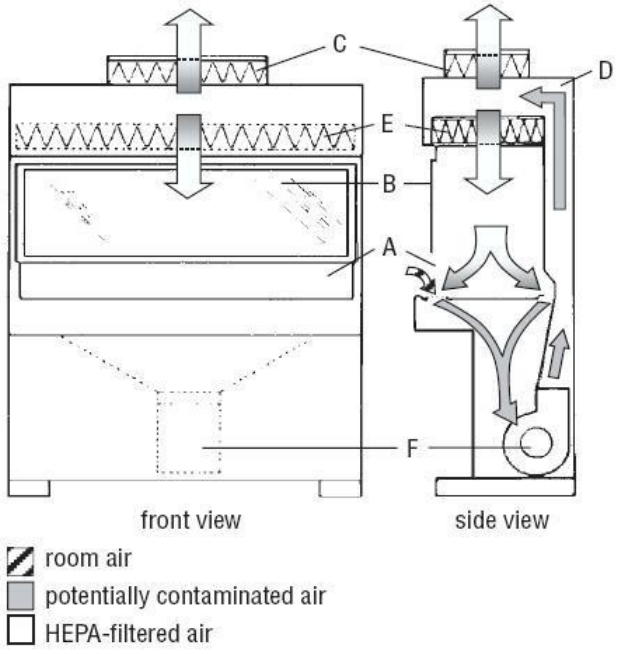
Biological Safety Cabinets

In the 1980s, cabinets with horizontal laminar flow were in widespread use. However, it was strongly recommended that they were replaced with vertical laminar air flow, as the horizontal flow of air was thought to direct airborne contamination into the breathing zone of the operator.[70-72]

BSCs are a type of vertical laminar flow cabinet. Air channelled downwards through the vents at the front and back of the cabinet creates a curtain of air, which protects the operator. Figure 22 on the following page shows a diagram of a BSC (front and side view) with arrows indicating the airflow pattern. The large opening in the front fascia allows access into and out of the cabinet and movement of the operator's arms working within. The effectiveness of the air barrier may be measured using the KI discus test method, as accepted by the European standard for Microbiological Safety Cabinets, EN 12469. This method involves applying a fine mist of potassium iodide droplets into the cabinet and measuring the portion of

escaped aerosol.[73] However, this test is not suitable for hazardous drugs, which may vary in physical state *i.e.* solid, liquid, or gas, and particle size.[24] BSCs are widely used in the United States and Germany.[73]

Figure 22. Airflow Diagram of a Biological Safety Cabinet (front and side view)



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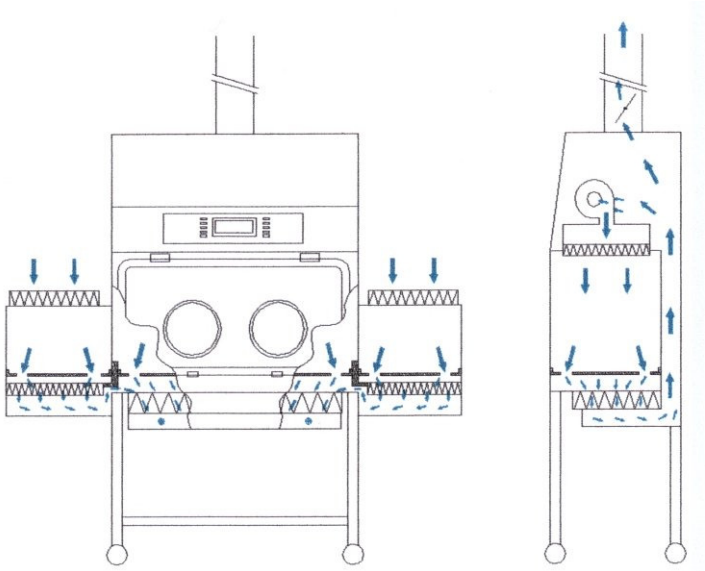
Isolators

Isolators are of a rigid or a flexible structure and are fully enclosed systems. They are designed to provide protection for the cytotoxic reconstitution and manipulation process from the outside environment. Operator access is *via* the front sleeve/glove port system, and introduction/removal from the isolator is *via* transfer hatches with interlocking doors. Isolators have built-in alarm systems warning of any system leakage or failure. See Figure 23 on the following page shows a diagram of an isolator (front and side view) with arrows indicating the airflow pattern.

Figure 23. Airflow Diagram of an Envair Model CDC-F Isolator (front and side view)



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Isolators are designed to operate under either positive or negative pressure. Negative-pressure will protect the operator in the event of a leak, and is favoured by the HSE,[24] as opposed to a positive environment which favours product protection and is supported by the MHRA.[74] The preferential pressure is debatable depending on favoured protection of the operator or the product.[55] A study comparing positive with negative-pressure isolators was not definitive regarding operator exposure.[26]

Isolator technology is used widely in Europe and Australia and significant integration has been seen into French and UK hospital pharmacy practices. Integration of the isolator was initially slow but it has superseded the conventional BSC,[74-76] and now approximately 90% of hospitals pharmacies use isolators in the UK.[75] Current practice in the UK favours the use of negative-pressure isolators.[24]

The air in both isolators (including hatches) and BSCs is exhausted and re-circulated *via* high-efficiency particulate air (HEPA) filters. These are glass fibre filters and are designed to trap particulate material of ≤2 μm from an air stream.[77]

However, levels of CP have been measured in the air while admixing in a BSC and in subsequent periods when no preparation was taking place.[34] The authors hypothesize that drug particles retained on the HEPA filter or present on surfaces within the BSC may be acting as a reservoir, thus, providing a source from which the molecules may vaporise slowly.[34] If this was a common occurrence, these filters would not be effective in retaining molecules of cytotoxic vapour, which would be smaller than the pore size of the HEPA filter. As a result, any cytotoxic aerosols generated would pass through the filter and re-enter the workroom, or in the case of air which is ducted externally, be released into the outside environment. It is therefore paramount to operator protection that whichever source is used, the air is exhausted externally away from the working environment.[4]

BSCs with vertical laminar flow were introduced as a superior measure of protective intervention, but with the development of more sensitive analytical methods it has been demonstrated that exposure is still apparent.[58;61;71] The downside of BSCs is that they are very sensitive to changes in air turbulence *e.g.* operator arm movements may disrupt the air barrier compromising operator and product protection. Removal of the operators gloved hands after working in the BSC is a potential source of contamination to the external environment. CP, 5-FU and MTX have been quantified in the air, on the floor and on workbenches in drug preparation areas when work had been carried out in a BSC.[28;58;61;64;66;71;78] Logically, isolator technology offering containment and either product or operator protection would appear to be more favourable. However, there is no firm evidence to support the superiority of isolators over BSCs. Isolators have limitations and there is the potential for contamination to pass through hatches from the main chamber to the outside environment. Cytotoxic contamination has been reported in both isolators and

BSCs.[61] In a comparative study, 83% of wipe samples taken from isolators tested positive compared to 8.3% from BSCs. Contamination on the finished product was also measured with the result that 35 out of 72 samples, compared to 3 out of 72 tested positive for cytotoxic contamination when prepared in isolators compared to BSCs, respectively.[61] These findings may reflect increased difficulty in cleaning isolator workstations.

1.6.2 Effectiveness of Personal Protective Clothing

Gloves

Gloves are the first line of protection against contact with cytotoxic drugs; therefore, it is important that the glove material is tested for permeability to these agents. The OSHA guidelines for the safe handling of chemotherapy agents concerning personal protection advise against the use of polyvinyl gloves and recommend the use of latex gloves.[47;79] However, issues have arisen over sensitivity to natural rubber latex with a possible allergic response.[80] The nitrile polymer is more resistant chemically, and is stronger than the latex polymer, but gloves made of latex are thicker.[38] Thick gloves may result in more discomfort during wear, whereas thinner gloves are preferable for dexterity and comfort.

Static permeability tests concluded that most gloves are impermeable or minimally permeable to cytotoxic drugs after certain periods of time.[38;81] Carmustine and paclitaxel were the only 2 of the 18 drugs tested which permeated through glove material. Of the 864 glove samples tested, only 3 showed evidence of permeation, one through a latex glove permeated by carmustine at 90 min, and two by paclitaxel, which permeated a polyurethane glove at 60 min and a neoprene glove at 120 min.[38] Alcohol 70% v/v, which is used regularly and sprayed liberally onto gloves did not aid the permeability of six cytotoxic drugs in commercially available brands of latex

and nitrile gloves.[82] Initially, these studies were encouraging. However, static tests are likely to underestimate the risk, and a study simulating latex glove usage demonstrated permeation, measured as breakthrough onto the cotton glove worn underneath when double gloving. CP easily penetrated the gloves after 10 min, followed by MTX after 62 min. The longer penetration time of MTX was attributed to its higher molecular weight (454.44 for MTX compared to 279.1 for CP).[30]

More rigorous tests using a device designed to subject the gloves to the dynamic conditions of rubbing, stretching and tension were carried out on 13 different gloves and 13 cytotoxic drugs. Permeation was related to exposure, and increased with time. The highest resistance to permeation was observed generally in latex gloves with a thickness of at least 0.24 mm.[83] The rate of drug permeation may be influenced by the thickness and composition of the glove, molecular weight and lipophilicity of the drug, the solvent into which the drug is dissolved, and the inactive ingredients in the drug formulation.[24] In actual practice, factors such as temperature,[84] humidity and pH of the skin may also have an influence.[83]

Glove materials vary between manufacturers and it is impossible to test every cytotoxic drug with every glove on the market. It is recommended that low-protein (<50 µg/g) powder-free gloves are used,[85] and double gloving carried out if it doesn't interfere with dexterity. Gloves should be changed every 30 min and replaced immediately if torn or punctured.[79] Special precautions to minimise glove contact should be adopted when working with low-molecular weight or lipophilic cytotoxic drugs *i.e.* nitrile gloves should not be used with etoposide,[57] as some sources have demonstrated permeability.[81] This may reflect the solubilisation aids present in the etoposide (vepeside) formulation.

Gowns

Gowns should be worn closed at the front with tight-fitting cuffs, which tuck underneath the gloves. They should be disposable, lightweight, low-lint and offer low permeability. The visible penetration (using fluorescein) and splash protection (beading on the gown surface) of six disposable gown materials with either water-based or non-water based cytotoxic drugs was investigated.[86] Polypropylene spun gowns were found not to be a sufficient barrier to the cytotoxics tested. Polyethylene or Saranax® (ethylene/vinyl acetate copolymer) gowns laminated with Tyvek® (spunbound polyethylene) were found to be the most protective barriers garments. The Saranax®/Tyvek® gown provided adequate splash protection and prevented penetration during a 1 min observation period of all 15 drugs tested.[86] The impermeability of this gown was also supported by another study which studied absorbance of 6 drugs after a 4 hour exposure time.[87] However, the least protective gowns were found to be more comfortable than those with polyethylene or vinyl coating. As protective garments increase in thickness, heat exchange by sweat evaporation is impeded leading to general discomfort - a factor that is important to ensure the gown is worn properly. Data are lacking to support the integrity of seams, and the protection provided during the preparation of large chemotherapy batches.

1.7 Methods for Monitoring Exposure to Cytotoxic Drugs

To identify the main exposure routes and to quantify potential health risks, biological and environmental monitoring are paramount to assess the occupational exposure risk to hospital personnel involved in the preparation and administration of cytotoxic drugs. The methods available to monitor exposure to cytotoxic drugs have been reviewed.[8;88;89] They can be divided into biological effect monitoring, biological monitoring and environmental monitoring.

1.7.1 Biological Effect Monitoring

Biological effect monitoring studies genotoxic, biological endpoints. Included in these methods are the cytogenetic methods sister chromatid exchange, chromosomal aberrations and micronuclei. These cytogenetic methods are non-selective, laborious and expensive, and the results of studies are conflicting. Several authors have found an increase in the frequency of these endpoints,[90-93] while others have failed to find a significant difference between workers and controls.[94;95]

Methods to detect DNA damage include point mutations and the Comet Assay.[96] A limited number of studies have been reported using these methods to detect DNA damage, so it is difficult to assess the usefulness of these tests. The Comet assay has been suggested as being a potential tool to measure for early genotoxic effects caused by exposure to cytotoxic drugs.[97] The first study reported using the Comet Assay to probe for induced DNA damage from cytotoxic drugs, showed nurses with an increase in DNA damage from the controls and less DNA damage in nurses using the required protection.[96]

1.7.2 Biological Monitoring

Biological monitoring methods can be either non-selective or compound selective.

Non-Selective Methods

Non-selective methods, for example urinary mutagenicity or thioether excretion, rely on the common properties of a group of chemicals to take measurements. Pharmacy personnel have tested positive for urinary mutagenicity, with the highest results recorded during the working week and subsiding to control levels when the exposure period decreased.[70] Another study found negative results.[98]

Many studies have been carried out using variations of the urinary mutagenicity test. In a review of 29 studies, 14 were found to be positive, and 15 negative for urinary mutagenicity associated with cytotoxic drug handling.[8]

The Ames test is used commonly to measure urinary mutagenicity by testing for mutagenesis in bacteria. Screening bacteria is a useful alternative to animal studies, but chemicals, which induce mutations in bacterial DNA, may not do so in human cells, therefore the test it is not a reliable predictor. The test is easy and not expensive to carry out, but lacks the sensitivity that would be required to detect the levels which may be absorbed by drug handlers occupationally, thus the suitability of the assay is questionable.[99]

Studies measuring thioether excretion in urine are contradictory. An increase in urinary thioether concentration was observed in nurses handling cytotoxic drugs.[100;101] Whereas another study of nurses concluded there was no relationship.[90]

The urinary mutagenicity and thioether excretion tests are limited as they can only be applied during the excretory period, generally 1 to 2 days.[8] Furthermore, positive results are not fully conclusive due to background genotoxic levels *i.e.* smoking, diet and environmental factors. In conclusion, these methods may not be sensitive enough or interpreted correctly. A negative test result indicating no exposure could be due to the lack of sensitivity of the method, whereas positive results may occur due to the lack of specificity of the method, creating unnecessary anxiety to the employee.[88;89]

Compound Selective Methods

Compound selective methods include sensitive chemical-analytical methods, which are used to quantify the parent drug or its metabolites typically from urine,

blood or environmental samples. These methods assure the sensitivity and specificity that has been lacking in biological methods. Typically, these methods are developed to quantify one analyte. A HPLC method for the simultaneous analysis of five cytotoxic agents was developed which could be applied to measure airborne levels and surface contamination.[102] Simultaneous analysis enables a broader risk evaluation to be carried out in a single analysis, although there is the risk of compromising of the sensitivity of the method.

Instrumentation used for this work includes; high-performance liquid-chromatography (HPLC) with either UV or fluorescence detection, often coupled or tripled with mass-spectroscopy to further improve the sensitivity; gas-chromatography coupled with nitrogen-phosphorus electron capture or mass-spectrometry; voltammetry inductively coupled with plasma mass-spectrometry (used commonly to determine urinary platinum).[66;103] Sensitivity is enhanced with liquid-liquid or solid-phase extraction which pre-concentrates and cleans-up the sample.[65] Derivatisation methods may also be used to increase the sensitivity and improve the selectivity of the method for the drug of interest.[104]

Using chemical-analytical methods, metabolites of cytotoxic drugs have been identified in blood and urine. α-fluoro-β-alanine, a metabolite of 5-FU was assayed for to measure 5-FU,[44] 6α-hydroxypaclitaxel and 3'-p-hydoxypaclitaxel were used to quantify levels of paclitaxel[105] and 4-hydroxycyclophosphamide was used as a measure for CP in plasma and urine.[30;106] CP (maximum concentration 0.76 μgL^{-1}), IFOS (maximum concentration 1.90 μgL^{-1}), DOX, EPI and platinum were found in the urine of 40% of pharmacy and hospital personnel in a German hospital,[27] and 5-FU and MTX tested positive in the urine of pharmaceutical plant workers for these two cytotoxic drugs.[44;107]

Given the large number of drugs used in cancer chemotherapy, testing has been limited to a few marker drugs, typically those in frequent use and for which validated assays are available. All conclusions reached so far are based on the capabilities of the experimental conditions and the limit of detection (LoD) or limit of quantification (LoQ) of the analytical method.

1.7.3 Environmental Monitoring

Environmental monitoring of cytotoxic drugs measures airborne contamination and contamination of surfaces, usually in the immediate environment. Chemical-analytical methods are used to quantify the levels of cytotoxic contamination after recovery from these areas.

Air Sampling

Air concentrations in cytotoxic drug preparation and administration areas have been measured by drawing environmental air through a filter and extracting the drug from the filter for analysis. The first data published on ambient air concentrations in preparation areas tested positive for environmental contamination of CP and 5-FU.[71] Studies have been carried out by means of filters of different materials *i.e.* PTFE, glass-fibre or filter papers, different filter pore sizes, and varying flow rates.[29-31;72] However, the concentration levels of the airborne contaminants, when present, were low in these studies, and may be due to the inefficiency of the method[4] or the vaporisation of one of the marker drugs *i.e.* CP from the filter.[35] Airborne CP was measured in a study where solid or liquid aerosols of CP were trapped by a glass fibre particle filter, and gaseous CP was frozen out in a cyrotrap.[34] This setup demonstrated that the air in the workroom contaminated with gaseous CP was higher than particulate CP. This may be due to the possibility that particles are sporadically

released and are therefore distributed less homogeneously in the air compared to gases.[35] It may also explain low levels of airborne particles found in previous studies.

Surface Wiping

Wipe sampling coupled with analysis by chemical-analytical methods has been widely used to measure contamination on a range of different surfaces. Typically, these methods employ a collection matrix and a solvent system to aid recovery of the drug. Pre-wetted and dry wipes, filters pre-moistened with ethyl acetate, cellulose pads moistened with water and cotton wool have all been applied as the collection matrix. Aqueous sodium hydroxide, aqueous ammonium acetate, water, ethyl acetate, methanol and hydrochloric acid have been used to aid collection of the drug from the surface, or as a desorbing solution in which to recover the drug.[20;28;29;42;58;66;108;109]

Considerable amounts of contamination have been recovered from surfaces in pharmacy drug preparation areas (75% of samples) and in drug administration areas (65% of samples) in a six site study carried out in Canada and the United States.[58] The levels of cytotoxic drug contamination, and the areas in drug preparation and administration where various studies have confirmed contamination, are documented in Table 1 on the following page. The reporting of the widespread contamination of surfaces makes the possibility of skin contact more likely.

Table 1. Levels of Cytotoxic Drug Contamination Recovered from Surfaces in Drug Preparation and Administration Areas

Area	Drug and Reference	Amount of Surface Contamination
Preparation Areas		
Inside the BSC	CP [28]	1 - 160 ng cm^{-2}
	MTX [28]	2 - 623 ng cm^{-2}
top side of BSC airfoil	5-FU [58]	110 ng cm^{-2}
bottom side of BSC grill	5-FU [58]	209 ng cm^{-2}
	CP [58]	66 ng cm^{-2}
	IFOS [58]	459 ng cm^{-2}
tray of BSC	MTX [109]	0.02 - 6 µg m^{-2}
Floor in front of BSC	5-FU [28]	48 - 236 µg cm^{-2}
Gloves	5-FU [28]	5 - 980 ng cm^{-2}
	CP [26]	100 - 5993 ng per glove
	IFOS [26]	99 - 1159 ng per glove
	MTX [26]	24 - 890 ng per glove
	MTX [109]	20 - 1900 µg per glove
Floor in preparation room	5-FU [58]	40.8 ng cm^{-2}
Floor outside preparation room	5-FU [58]	2.31 ng cm^{-2}
Floor in preparation room where an isolator was used for preparation	Pt [26]	5 - 130 ng cm^{-2}
	CP [26]	625 - 1596 ng cm^{-2}
	IFOS [26]	61 - 1503 ng cm^{-2}
	MTX [26]	20 - 674 ng cm^{-2}
Sink	CP [20]	<0.005 ng cm^{-2}
	5-FU [20]	<0.98 ng cm^{-2}
Fridge door handles	CP [20]	<0.002 ng cm^{-2}
	5-FU [20]	< 4.3 ng cm^{-2}
Waste bin	CP [66]	1286 pg cm^{-2}
	Pt [66]	2700 pg cm^{-2}
	IFOS [66]	28 pg cm^{-2}
Storage shelves	CP [66]	5106 pg cm^{-2}
	Pt [66]	2700 pg cm^{-2}
	IFOS [66]	1860 pg cm^{-2}
Telephone	MTX [108]	11 µg m^{-2}
Administration Areas		
Floor	CP [8]	<0.02 - 4.5 µg cm^{-2}
Tables	CP [8]	0.2 - 4.5 µg
	5-FU [8]	4.9 - 22 µg

Pt = platinum from platinum-containing compounds *i.e.* carboplatin and cisplatin

Immersion/Rinsing Techniques

The application of immersion techniques, coupled with analysis by chemical-analytical methods, has enabled cytotoxic residues on the surfaces of cytotoxic drug vials to be quantified.[41-43] Typically, this involves rinsing the object or immersing it in a suitable solvent, with agitation for a defined period of time, followed by collection and analysis of the solution. Immersion and rotation of stoppered vials in water for 30 sec was adequate to remove and measure 5-FU, etoposide, CP, IFOS, DOX and docetaxel contamination on the external surface of drug vials. This same method was effective in measuring cytotoxic contamination on packaging.[41] Potassium hydroxide (0.01 M) has also been used as a rinsing/immersion solution to recover the external surface contamination of 5-FU on vials of the corresponding drug.[40]

Immersion techniques are also applied to measure cytotoxic contamination on gloves. Typically, the gloves are placed into a container of desorbing solution and agitated for a defined period of time, followed by collection and analysis of the solution.[28;68]

Solvents selected to aid collection of the drug from a surface and desorption of the drug into a suitable desorbing solution are likely to be drug specific. The solvent chosen depends on the solubility of the drug, pKa values, solvent pH, stability in the solvent and solvent compatibility with the analytical method.

1.8 Removal of Contamination and Degradation/Inactivation of Cytotoxic Drugs

The effectiveness of removing cytotoxic residues from surfaces during cleaning is not often considered. Ideally, cleaning after the manipulation of cytotoxic drugs should involve the physical removal of drug contamination from a non-disposable surface to a disposable surface and drug breakdown into less toxic

compounds.[35] NIOSH recommends that all surfaces are cleaned according to a protocol that includes an appropriate detergent if available, which may be a deactivation agent.[4] The agent used should preferably remove/breakdown biological and chemical contamination,[4] and be compatible with the surfaces to which it is applied.[110]

Cytotoxic drugs represent a diverse range of chemical structures and OSHA recognises that there is no single accepted method of chemical deactivation for all cytotoxic drugs used currently.[79] Previous methods have involved the use of acid and strong oxidising agents,[111;112] at concentrations which are too harsh for use on sensitive equipment in the hospital environment.[113] In 1985, the IARC included cytotoxic drugs in its program for the treatment of contaminated waste.[114] Studies carried out to investigate the efficacy of oxidising agents at acceptable concentrations *i.e.* sodium hypochlorite (5.25%), liquid hydrogen peroxide (≤30%) and Fenton reagent (30%) were suggested as part of a program to assess the efficacy of oxidation in degrading 36 cytotoxic agents.[113] Sodium hypochlorite (5.25%) was efficient at degrading CP, melphalan, idarubicin, DOX, EPI, aclarubicin and daunorubicin to non-mutagenic residues above the LoD.[113;115;116] Liquid hydrogen peroxide (≤30%) was also efficient in degrading CP, but less efficient in degrading DOX.[113;115] Treatment of DOX with sodium hypochlorite and Fenton reagent was more successful with >99.96% efficiency achieved in the same experiment.[115]

1.9 Closed-System Containment Device

Prevention of the primary contaminating event is more effective than removing the contamination once it has occurred, and will also prevent the transfer of secondary contamination.[20] To prevent the primary contaminating event, NIOSH recommends the use of a closed-system drug transfer device to prepare cytotoxic

drugs. It defines a closed-system device as "a transfer-device which mechanically prohibits the transfer of environmental contaminants into the system and the escape of hazardous drug or vapour concentrations outside the system".[4] This is also supported by ISOPP, which includes in its guidelines (awaiting publication) that the system should be 'leak-proof' and 'airtight'.[52]

There are a number of products available commercially that declare safe drug-transfer *i.e.* the Alaris Smart Site®, the Tevadaptor Vial™ Adaptor System, the Chemo Mini-Spike Plus™ Dispensing Pin, the Chemoprotect Spike®, and the PhaSeal® Protector 50 and Injector Luer-Lok. These five devices were tested to determine how effectively they contained vapour produced from the over-pressurization of a drug vial. Titanium tetrachloride gas generates a visible smoke and expands when it comes in contact with moisture in the air, thus moist air was injected into vials containing titanium tetrachloride to simulate gas-containing active drug into the environment.[117;118] In comparison with the four other drug-transfer systems, the PhaSeal® system was the only one to prevent titanium gas leakage, concluding that it was the only device to offer a closed-system.[117;118] Titanium tetrachloride was used in this study to simulate drug reconstitution as it has a molecular weight similar to 5-FU and CP.[118] However, there has been some debate between the manufacturers' of the Tevadaptor Vial™ and the supervisor of the study, regarding the validity of the study and the effectiveness of titanium tetrachloride as a substitute for a cytotoxic drug vapour.[119-121] The Tevadaptor™, Alaris® System and PhaSeal® system were tested further for containment (unpublished work) when simulating cytotoxic preparation and administration using a fluorescent dye solution. No leakage was observed when using the PhaSeal® device, but it was observed when using the Tevadaptor™ and the Alaris® System.[122] In-house testing by Teva (unpublished work) using wipe sampling

and radioactive analysis of possible leakage demonstrated the Tevadaptor™ to be effective.[123] Regardless of these various studies, the effectiveness of only one closed-system device - the PhaSeal® device in reducing surface contamination has been published in peer-reviewed scientific journals.[20;67;78;124;125]

A number of studies have been carried out comparing usage of the PhaSeal® device with standard reconstitution procedures.[32;62;67;124] As a result of the findings of surface contamination in six cancer treatment centres in the United States and Canada[58] the PhaSeal® device was evaluated in the a Cancer Center (U.S.), which at the time had been completely rebuilt with new equipment. The study demonstrated a significant reduction in surface contamination of CP and IFOS.[78] The device has been investigated in research centres worldwide, including the MD Anderson Cancer Center, Houston, USA,[78] Uz-Gent University Hospital, Belgium,[67] University Hospital of Utah, Salt Lake City, USA[126] and Japan.[64] All these studies have supported a significant reduction of contamination when using the PhaSeal® device, *i.e.* in the Belgium hospital a 10-fold decrease in environmental contamination was observed when comparing the traditional method.[67]

A long-term study carried out in an outpatient oncology clinic in a Swedish hospital showed virtually no detectable environmental contamination after using the PhaSeal® device to prepare and administer CP and 5-FU over a period of 1 year.[20] The study was designed so that the drugs were prepared outside of a clean-room environment *i.e.* on the bench top in the drug preparation room without the use of a BSC. The staff involved in the study considered the PhaSeal® device easier and more efficient to use on the bench top than in a BSC. The turnover for preparation and administration of the cytotoxic drug was shorter due to the reduction of personal protective measures required to work in a clean-room, which are time consuming to

don and are expensive.[20] However, this lack of protection in the form of ventilation would not be acceptable under UK standards of practice, which require that sterile dosage forms are manufactured providing optimal operator and product protection.

The prevention of drug leakage into the environment has been observed visually when using the PhaSeal® device and a fluorescent marker for the following phases of drug handling: reconstitution of a dry powder, drug transfer from the vial, simulated drug administration, and IV push administration through an IV port.[32]

The design features of the PhaSeal® device that creates a closed-system are an expansion chamber that neutralizes over and under-pressure in the drug vial, and a double membrane that forms dry connections when the components are fitted together.

More than 500 cancer centres, oncology practices and speciality pharmacies in the United States use the PhaSeal® device for the handling of cytotoxics.[127] The device appears to be an effective answer to the containment of hazardous drugs as referred to in the NIOSH alert. It is designed not only to maintain asepsis, but also to protect the operator and personnel who administer the drug. The benefits of the closed-system are also maintained during the process of waste handling, as the device is disposed of in the normal chemotherapy waste, without dismantling the components.

1.10 Selection of Cytotoxic Marker Drugs

For the purpose of this work, some of the cytotoxic drugs used most widely and clinically were selected to be used as marker drugs. Marker drugs should be drugs of high significance and of frequent use, to provide an initial estimation of the risk. Personnel working with cytotoxic drugs may be exposed to a cocktail of different drugs, all of differing levels of toxicity. Therefore, marker drugs from

different chemical classes were selected. The results of all determinations with these drugs are influenced strongly by the LoD of the analytical method applied.

CP, 5-FU, MTX, DOX and EPI were the marker drugs selected for studies reported in this thesis. More in-depth studies of these drugs have been made, reviewing the chemical properties, for the purpose of including them in the experimental part of this thesis.

1.10.1 Cyclophosphamide

CP (2-[*bis*(2-chloroethyl)amino]-perhydro-1,3,2,oxaza-phosphorine-2-oxide monohydrate) is one of the most used common bifunctional alkylating agents.[8] The chemical structure of CP is shown in Figure 2 (page 4). It is supplied as a white powder for reconstitution with water for injections (WFI). It may be infused in 0.9% normal saline (NS) or 5% glucose.[24] The degradation of CP occurs primarily by hydrolysis with temperatures above 30ºC accelerating this breakdown. Consequently, it is recommended that CP products are not stored above 25ºC.[128] The rate of decomposition is independent of pH over the range of 2 to 10. Outside of this range acidic and basic catalysis may occur.[128] Under acidic conditions, the pathway and breakdown products formed depend upon the pH of the solution, either route will produce phosphoric acid, propanolamine and nor-nitrogen mustard.[5] Under basic or neutral conditions, hydrolysis occurs by an initial intra-molecular alkylation, forming hydrochloric acid and a bicyclic compound.[5] However, this compound is very labile in aqueous solution and may breakdown to further products.[5]

Contamination of CP in pharmacy preparation and administration areas has been reported to a large extent[26;58;62;63;67;68;78;124;125] - see Table 1 (page 42). This is of great concern, as CP has been classified by the IARC as Group 1 *i.e.* carcinogenic to humans.[9] CP is an oxasophosphorine prodrug which undergoes enzymatic oxidation

in the liver to form 4-hydroxycyclophosphamide and its tautomeric form, aldophosphamide.[128] 4-hydroxycyclophosphamide is further converted to 4-ketocyclophosphamide, and aldophosphamide to carboxyphosphosphamide, both are non-cytotoxic *in vivo* or *in vitro*.[1] A β-elimination reaction converts aldophosphamide to phosphoramide mustard, which is a bifunctional alkylator of DNA, and acrolein which has been associated with bladder toxicity.[8]

Structurally, CP does not possess a strongly conjugated chromophore,[129] therefore some analytical methods are not very sensitive, and it is necessary to monitor at low UV wavelengths where there might be the possibility of other compounds absorbing and interfering with the assay.

Potassium hydroxide, in methanol (0.2 M) solution for 1 hour, or sodium hypochlorite (5%) solution for 24 hrs is recommended for the chemical degradation of CP.[24]

1.10.2 5-Fluorouracil

5-FU (5-fluoro-2, 4(1H, 3H) pyrimidine-dione) is a six-membered fluorinated pyrimidine of the antimetabolite family - see Figure 8 (page 8) for the chemical structure of 5-FU. 5-FU is stable at acidic pH,[24] although precipitation may occur at a pH of less than 8.0, depending upon the concentration.[128] It may be subject to alkaline hydrolysis, the rate increasing above pH 9.0, therefore it is formulated within the pH range of pH 8.0 - 9.0 by the addition of sodium hydroxide.[24] 5-FU may be administered undiluted, or diluted as an infusion in NS, WFI or 5% glucose.[24] When prepared in these diluents, thermal and photochemical exposure may cause the breaking of the chemical bonds of the pyrimidine ring between N3 and C4, and N1 and C5 to produce urea.[24] Moreover, the most likely method of degradation is alkaline hydrolysis, which is slow. This leads to the production of barbituric acid,

which further degrades to urea.[24] The drug is stable at acid pH, but its solubility is compromised and precipitation may occur. The solution is normally colourless to faint yellow, due to the presence of free fluorine. A dark yellow colour of the solution is indicative of decomposition.[128]

5-FU has been classified by the IARC as Group 3 *i.e.* not carcinogenic to humans but mutagenic and teratogenic.[9] 5-FU contamination has been widely reported in pharmacy preparation and administration areas[20;26;45;61;62;66;71;125] – see Table 1 (page 42). However, to date there is no satisfactory method for the degradation of 5-FU *in situ* with the aim of decontamination.

1.10.3 Methotrexate

MTX (4-amino-N-methyl) is a 2,4-diamino-substituted pteridine ring linked to a p-aminobenzoyl portion, which is amine bonded to a glutamic acid unit - see Figure 10 (page 10) for the chemical structure of MTX. MTX is supplied as a clear yellowish solution for injection or infusion which may be diluted in NS or 5% glucose.[24] Like 5-FU, sodium hydroxide is added to the formulation to adjust the pH between 8 and 9.[24] MTX is relatively stable in aqueous solution but its solubility is poor and is pH-dependent. It is more soluble in a solution of mineral acids and in dilute solutions of alkali hydroxides and carbonates.[24] Maximum stability is observed between pH 6.6 – 8.2, although it is subject to hydrolysis and photolysis, the rate increasing with increasing pH.[24;128] For this reason it is recommended that MTX preparations are stored between 15ºC to 30ºC, and protected from light.[24]

MTX has been classified by the IARC as Group 3 *i.e.* not carcinogenic to humans but mutagenic and teratogenic.[9] MTX has increasingly wide clinical applications not only in oncology chemotherapy, but also as an immunosuppressive agent,[130] hence the presence of MTX contamination in the environment. It has been

measured on vials, gloves, the handles and doors of storage fridges, shelves, on the floor, telephone hand-sets, bench areas, trays and areas inside BSCs[26;29;45;108;109] – see Table 1 (page 42).

1.10.4 Doxorubicin

DOX (8S,10S)-10-(4-amino-5-hydroxy-6-methyl-tetrahydro-2Hpyran 2-yloxy)-6,8,11-trihydroxy-8-(2-hydroxyacetyl)-1-methoxy-7,8,9,10 tetrahydrotetracene-5,12-dione) consists of an amino sugar (daunosamine) linked through a glycosidic bond to a tetracyclic aglycone (doxorubicinone).[24] Aqueous solutions of DOX are yellow/orange at acid pHs, and violet blues at pHs above pH 9.[131]

DOX hydrochloride is presented either as a lyophilized product in a rapid dissolution formula that requires reconstitution in NS or WFI, or as a 2 mg mL^{-1} solution for injection.[24] The lyophilized product should be stored at room temperature and protected from light. Once reconstituted DOX is stable for up to 48 hrs at room temperature under normal light.[24] The solution for injection should be stored under refrigerated conditions and protected from light.[128] DOX exhibits pH-dependent stability in solution. Maximum stability has been reported to be in the pH range of pH 4 to 5.[24;128] DOX is unstable at pH outside the range of pH 3 - 7.[128] In acidic media below pH 3, splitting of the glycosidic bond produces a red-coloured water insoluble aglycone, and a water-soluble amino sugar. In alkaline media a colour change from red to purple is indicative of rapid degradation.[24;132]

1.10.5 Epirubicin

EPI (10-(4-amino-5-hydroxy-6-methyl-oxan-2-yl) oxy-6,8,11-trihydroxy-8-(2-hydroxyacetyl)-1-methoxy-9,10-dihydro-7H-tetracene-5,12-dione) is the 4'-epimer

of DOX. It consists of the tetracyclic aglycone (doxorubicinone) linked to an amino sugar (acosamine) *via* a glycosidic bond.[24]

Similar to DOX, EPI is supplied either as a lyophilized product or as a solution for injection, which requires reconstitution in NS or WFI. It also exhibits a similar pH stability profile to DOX.[24;128] Both DOX and EPI are highly coloured fluorescent compounds and are therefore easy to detect *i.e.* the native fluorescent of DOX has been used as a tool to detect contamination of DOX on the skin.[116]

The anthracyclines are best represented by DOX or EPI and have been classified as Group 2A by the IARC *i.e.* probably carcinogenic to humans.[9] See Figures 13 and 14 (page 12) for the chemical structures of DOX and EPI, respectively. Analytical methods are numerous for the separation of these compounds however, environmental contamination studies of DOX and EPI are limited.[64;71]

Soaking in dilute sodium hypochlorite (1% available chlorine), followed by water is recommended for the treatment of spillages or leakages of DOX or EPI.[133]

1.11 Introduction to this Study

There is plentiful evidence in the literature demonstrating that cytotoxic contamination released from the manipulation and administration of cytotoxic drugs in practice does occur. Even with the improvement of personal protective measures recommended by guidelines, measurable levels of cytotoxic drugs have still been detected in the urine of personnel and in the facilities where these drugs are prepared and administered.[25-30;61;63;66;69;108;109;125] There are many sources and routes of exposure, not all of which can be avoided in current practice. Contamination of cytotoxic drugs is inconsistent and cannot be predicted. Therefore, intervention is needed to effectively remove the contamination or to prevent its occurrence.

The following research questions were raised during the literature review:

- There is no standard recommended method of decontamination and cleaning after the manipulation of cytotoxic drugs, and guidelines are brief. What decontamination and cleaning procedures are being used in areas where cytotoxic drugs are being manipulated? What methods and products are being used?

- How were these procedures developed? Are they effective? Are they validated? Are they just concerned with removing biological contamination *e.g.* microorganisms or is chemical contamination also addressed?

- If cytotoxic contamination is a concern, how deep are these concerns? Do managers and operators of cytotoxic reconstitution services recognise these concerns?

- Practically, can cytotoxic contamination be removed by current decontamination procedures or be broken down into less harmful products?

- How much contamination is actually present in an isolator workstation? Access for cleaning the work area of an isolator can sometimes be difficult.[134] Can cytotoxic contamination be removed by cleaning or is it persistent? The isolator may contain the contamination, but when products are removed from the isolator is it transferred outside *via* surface contamination on the finished product, thus spreading contamination to other environments? Is there also the risk of product cross-contamination occurring in the isolator?

- What are the sources of cytotoxic contamination? Can contamination be prevented by using a closed-system device *i.e.* the PhaSeal® device? How effective is this device in reducing contamination inside the isolator and on the finished product leaving the isolator?

Overall, the aims of this work were to identify deficiencies in current practice, to reduce cytotoxic contamination in the working environment and also on the surfaces of pharmaceutical dosage forms supplied to wards, clinics and patients.

1.12 Project Structure

In order to investigate systems to reduce the risks of occupational exposure, a three-part project was devised.

The first phase involved researching the current decontamination methods applied in cytotoxic reconstitution units in UK hospitals, which manipulate cytotoxic drugs. This required sending out a questionnaire to reconstitution service managers in NHS hospital to gain information about the decontamination procedures and products used.

The second phase involved the evaluation of practical methods to remove and degrade cytotoxic contamination on surfaces in an isolator workstation, which could be applied as a decontamination protocol.

The third phase investigated the prevention of contamination in an isolator by using a closed-system device for fluid-transfer.

It was envisaged that results from these studies could inform the practice of cytotoxic reconstitution and manipulation and would encourage the adoption of standard, validated techniques to minimise occupational exposure to cytotoxic drugs. Although this work is focused on pharmacy reconstitution centres, by reducing contamination on the external surfaces of finished products, benefit to personnel working in clinics or on hospital wards would also be realised.

2. Investigation into Decontamination Procedures Carried out after the Manipulation of Cytotoxic Drugs

2.1 Introduction

Decontamination may be defined as the use of physical and/or chemical means to render a surface or item safe for handling, use or disposal.[110] This can refer to both biological and chemical decontamination, which are paramount in the safe preparation of cytotoxic drugs, to maintain product and operator protection. Decontamination is, in practice a combination of cleaning and disinfection/sterilisation. These are different processes but may be combined, although generally they are antimicrobial processes, which do not address chemical decontamination. Chemical decontamination may be achieved by physical removal, degradation or neutralisation.[110]

It was apparent from the literature that there are no cleaning standards for cytotoxic contamination removal; the available guidelines are very brief and not definitive.[4;47;49-51;135] A review of cleaning procedures is required to find out the procedures that are being used in facilities in the UK, which compound cytotoxic drugs.

2.2 Objectives

The objectives of this study were to investigate the cleaning and disinfection procedures that are being applied to decontaminate the areas used for the compounding of cytotoxic drugs. To achieve this, a questionnaire was designed and sent to NHS hospital pharmacy Aseptic Services Units (ASUs) in England, Wales and Scotland where cytotoxic drugs are compounded.

2.3 Study Design

2.3.1 Design of the Questionnaire

To achieve the objectives of this study, data would need to be collected from a potentially large number of respondents, covering a large geographical area. Questionnaire was selected as the tool for collecting data as it has advantages over other data collection methods *i.e.* interviews or focus groups. Questionnaires are easier to administer confidentially, and if confidentiality is offered participants are likely to respond more honestly. They are also inexpensive to administer, and if the questionnaire is distributed *via* email, potentially the response time may be quicker than setting up an interview *i.e.* arranging the interview and the time taken for travel.

The questionnaire was designed after literature review, knowledge of practice, visit to an ASU, and consultation with industry manufacturers concerning cleaning procedures and products. A pharmacy manager of an ASU, who is a member of the Pharmaceutical Aseptic Services Committee, piloted the questionnaire. This was to check the relevance of the questions, ascertain if the questionnaire was unambiguous, and determine the time taken to complete.

The questionnaire comprised of ten questions, of which nine were closed and one was an open question. Closed questions gave the respondent a restricted choice of answers, and requested the respondent to select an answer from the specified response options. These questions were easier and quicker to answer, provided consistency, were easier to code, and analyse. Whereas the open question gave the respondents the opportunity to answer the question in their own words, allowing the respondent to record any issues not covered in the questionnaire. Many of the closed questions were also accompanied by a section to record any 'other' information, which was not covered in the specified response options. The title of the

questionnaire was 'Investigation into the Decontamination (cleaning and disinfection) Procedures Carried out after the Manipulation of Cytotoxic Drugs.'

Prior to the start of the questionnaire, the name of the hospital was requested. The questions and the rationale for inclusion in the questionnaire are given below:

Question 1. In your hospital, what type of workstation do you use for the compounding of cytotoxic drugs?

This was asked to identify the main type of containment equipment used for the compounding of cytotoxic drugs in the hospital facility *i.e.* in an isolator (negative or positive) or in a BSC (ducted internally or externally). The option of stating other areas was included. The answers to the following questions 2 to 8 were concerned with the main response to this question.

Question 2. How would you describe your concerns about cytotoxic contamination in this area?

This was designed to ascertain how concerned the participant was about cytotoxic contamination. Participants were asked to select only one of the four possible responses *i.e.*
'extremely concerned,' 'slightly concerned,' 'indifferent' or 'not concerned at all.'

Question 3. Do you apply a cleaning and disinfection procedure to this area?

Here, the option was given to select 'a cleaning procedure,' 'a disinfection procedure,' or both combined *i.e.* 'a cleaning and disinfection procedure.' The choice of answer to this question determined which question the responded should answer next - , either question 4 or 5.

Question 4. Which of the following do you use for the cleaning of this area?

Participants were asked to identify from the list provided which, if any, of the water quality or alcohol preparations listed that they used for the cleaning of the area. They were also asked to provide details of the type of detergent, if used, for cleaning and select from the drop down menu the frequency of use, 'daily', 'weekly' or 'monthly'.

Question 5. Which of the following do you use for the disinfection of this area?

Participants were asked to identify from the list provided which liquid biocides or gassing/fumigation processes they used for the disinfection of this area. The common liquid biocides, according to manufacturers advice and literature review were listed *i.e.* alcohol, stabilised chlorine dioxide, quaternary ammonium compounds (QACs), QACs/stabilised chlorine dioxide blends, sodium hypochlorite, hydrogen peroxide, stabilised glutaraldehyde and chlorhexidine.[110] Formaldehyde, hydrogen peroxide, peracetic acid[24] and ozone gas were the options given as gassing/fumigation processes.[24;110] There was the option to include 'others', which may not have been listed.

For any biocides selected, the method of application *i.e.* impregnated wipes, spray with dry wipes, or liquid with dry wipes, was requested from a drop down list. The frequency of use of the biocides or the fumigants selected, 'daily', 'weekly', 'monthly' or 'yearly' was also asked for from a drop down list.

Question 6. Does the decontamination procedure for this area differ depending upon which drugs have been manipulated?

The option of answering 'yes' or 'no' was provided. If 'yes' was chosen details were requested.

Question 7. In your opinion which of the following best describes the purpose for the decontamination procedure which you, have described?

The respondents were asked to select only one of four options *i.e.* 'to remove cytotoxic contamination'; 'to disinfect/sterilise the area'; 'to maintain a sterile environment and remove some cytotoxic contamination'; or 'to maintain a sterile environment and remove all cytotoxic contamination.'

Question 8. How was the decontamination procedure you use devised?

Information concerning where the procedure had been derived from was sought. The options were 'national/international guidelines', 'literature or references', 'other facilities or manufactures advice'. There was the option of including other sources, which were not listed.

Question 9. Have you ever measured for cytotoxic contamination in the following areas, and if so was any cytotoxic contamination found?

The areas listed were vials, the finished product, airborne, gloves and surfaces inside or outside the isolator. There was the option of adding other areas not listed. If contamination had been measured for, it was asked to confirm either 'yes' or 'no' if it had been found.

Question 10. Please comment on any concerns you have about manipulating cytotoxic drugs and any problems, which you have experienced?

This was an open question, which gave the respondents the opportunity to describe any concerns, or in their experience, any relevant problems they may have encountered.

2.3.2 Ethics Committee Approval

In 1964, the 'World Association Declaration of Helsinki' originally outlined ethical principles for researchers in medical research. The declaration is the definitive code detailing ethical practice in health related research. Included within its principles is the freedom of the individual to decide upon participation, the need for consent to be informed and minimisation of the risks to the individual balanced against the need for research. Within the healthcare environment, these principles are assured by NHS Ethics Committee assessment of work before it is conducted. Research Ethics Committees (RECs) assess the safety, appropriateness and quality of the proposed research in the context of adherence to the ethical principles outlined above.

The taxonomy of the questionnaire was questionable. Classification as a 'Service Evaluation' or as an 'Audit' would not require ethical approval. On the other hand, classification as 'Research' would require ethical review. Guidance was sought from the Central Office for Research for Ethics Committee (COREC) as how to classify the questionnaire. The response was that the question was classified as a Service Evaluation.[136] However, their taxonomy is not watertight and conflicting opinions were received. Some host organisations *i.e.* NHS hospital Trusts considered an independent ethical review to be essential as NHS staff were involved, and after an application was made to an NHS REC, the REC itself considered the questionnaire to be classified as 'Research', conflicting the opinion of its organising body.

As more than one participant from more than one site would be involved, an application was made to a MREC (Multi-centre Research Ethics Committee). The

application for Version 1 of the questionnaire was made to the South West Devon REC.

Ethical opinion was favourable to carry out the research at NHS Hospital Trusts in the UK. The Approval Letter (REC reference number: 06/Q2103/120) from the South West Devon REC can be found in Appendix 1. The REC suggested that the request for the participants' name and job title were removed from the questionnaire to assist in maintaining confidentiality. This was amended and Version 2 was issued. It should be noted that the questionnaire was designed to be sent electronically, therefore some of the options, which were in the form of a drop down menu, are not apparent on the paper version, which can be found in Appendix 2.

2.3.3 Research Governance Approval

In addition to the MREC application, all activities classified as research within the NHS must comply with the Department of Health Research Governance Framework and be approved formally by individual Hospital Trust Management Committees within the Trust. Such committees review all research carried out in the Trusts facilities to ensure NHS resources are used favourably to support research. This involves a number of internal authorisation processes and exchanges between the R&D manager, principal investigator, participant, finance departments, and data protection specialists.

The NHS in England is managed locally by ten Strategic Health Authorities (SHAs), which are separated geographically into the North West, North East, Yorkshire and the Humber, West Midlands, East Midlands, East of England, London, South Central, South East Coast and South West. The aforementioned SHAs, Wales and equivalent departments in Scotland were applied to. Within each

SHA, the NHS is split into Trusts, some of which are responsible for managing acute NHS hospitals and others are responsible for the primary care sector.

All relevant acute hospital Trusts within the UK were approached to obtain research governance approval prior to commencement of the research. The details of the R&D contact for each Trust was obtained from a list provided by the NHS R&D forum. This totalled approximately 181 hospital Trusts.[137] R&D applications were made to comply with the procedures from each Trust. The procedure was different for each Trust. R&D applications in the South West and Scotland SHAs operated on a Single Point of Entry, where only one application was necessary to cover all Trusts in that SHA. In some cases, the committee chair was able to grant approval without consultation with the committee, or the questionnaire was classified as a Service Evaluation and approval was not required.

The national standard R&D form, which was the recommended form at the time, was accepted by most Trusts. Others would not accept the standard form and had their own, often-complicated R&D form.

2.3.4 Targets and Population Size Selection

Pharmacy managers in charge of ASUs, which prepare cytotoxic drugs centrally in NHS hospitals, were targeted as potential participants for the research. The number of NHS hospitals providing this service in the UK determined the population size. An accurate figure of the population size could not be obtained, but was anticipated to be approximately 181, as this is the approximate number of hospital trusts in the UK.[137] Willingness to participate was a major factor in determining a representative population. To encourage participation the questionnaire was designed to take up a short amount of time *i.e.* approximately 15 minutes, causing as little inconvenience as possible.

A 'Letter of Invitation' (see Appendix 3) was included with the questionnaire to promote the research, give clear instructions and to express anonymity. Basic ethical principles were applied to the NHS staff participants. The questionnaire was expressed as being non-compulsory and a response to the questionnaire would be taken as consent to participate.

2.3.5 Method of Distribution

After careful consideration, the questionnaire was distributed electronically. Electronic methods have shown a high degree of acceptance (57%) versus the conventional paper version (13%), and the benefits of completeness of data, speed of data flow and data handling workload using this method have been demonstrated.[138]

There is no central list of NHS aseptic service managers available, and the NHS Trust websites were not forthcoming with contact details of the appropriate individuals. The Chemist and Druggist Directory provides a register of NHS Hospital Trusts,[139] however the details given were not specific for ASU pharmacy managers. The British Oncology Pharmacy Association (BOPA) was approached, however they advised that they would not be an ideal route of circulation as there were too many miscellaneous members, and recommended that the relevant targets were identified through Specialist Pharmacy Interest Groups concerning cytotoxic and aseptic handling procedures.[140] The National Pharmaceutical Production Committee was approached and agreed to assist in its distribution. The questionnaire was distributed *via* a cascade system. It was sent to the chairs and secretaries of the following local groups with the request that it was forwarded to members who were ASU managers; Yorkshire Technical Services Group, North-East Quality Assurance Production and Preparation Group, South Thames Aseptic Services Managers, London and South East Licensed Production Managers, West Midlands Technical Pharmacy Group,

Eastern Technical Strategy Group, Eastern Preparative Services Network Group, Sussex and Hants Technical Services Group, South and West Technical Group, Thames Valley Technical Group, North Thames and Kent Aseptic Managers Group, North West Aseptic Services Managers, Welsh Association of Production Pharmacists, Scottish Aseptic Specialist Interest Group and Scottish Quality Assurance Interest Group. It was also requested that the researcher was copied on all emails so that the response rate could be determined.

2.3.6 Method of Response to the Questionnaire

The participants were given the option of either completing the questionnaire electronically and returning it *via* email, or printing off a copy, filling it out by hand and returning it *via* FREEPOST.

The name of the hospital was requested in the questionnaire so that responses could be coded. The intention was that after 4 weeks, a reminder would be sent out to non-responders using the same method. A second reminder would follow this up to non-responders 4 weeks later. The chairs and secretaries of the special interest groups would be informed of who had responded to avoid unnecessary follow-up. After this time, non-responders were considered as 'unwilling to participate' and no further contact would be made.

2.3.7 Data Recording and Analysis

Electronic responses were stored on a password protected secure computer hard drive. Paper responses and a paper copy of the electronic responses were kept in a secure cabinet in a locked room. The responses were coded chronologically upon receipt and according to each SHA.

The results were entered into the computer software SPSS (Statistical Package for Social Scientists), Version 14.0 (SPSS Inc. Chicago, Illinois, U.S.). Themes and trends from the results were explored and analysed using descriptive statistics.

2.4 Results

2.4.1 Conduct of Research

Version 2 (see Appendix 2) of the questionnaire was distributed electronically to ASU managers. This kept the handling workload for the Specialist Pharmacy Interest Groups, who were distributing the questionnaire, to a minimum and enabled rapid distribution. The questionnaire was sent knowingly to 115 ASU pharmacy managers however, it was forwarded on to other email addresses without copying the researcher, thus the exact number of recipients could not be calculated.

2.4.2 Participant Response

The questionnaire was distributed once, following the decision not to follow up to non-responders after 4 weeks. To assist in maintaining confidentiality, it was advised by the REC, that the option for the participant to provide their name on the questionnaire was removed. This made the participants who responded using the paper method and wished to keep the name of the hospital confidential, non-identifiable. Therefore, details *i.e.* hospital name or email address could not be given to the distributors of the questionnaire to eliminate these responders from the follow-up list. Resending of the questionnaire may have caused unnecessary distribution to responders and to those who had already decided not to participate. In total, 44 responses to the questionnaire were received, 62.8% of the responses were electronic and 37.2% were paper responses.

2.4.2.1 Demographics

The number of responses from each SHA was determined and is presented in Table 2 below. The geographical origin of only one response could not be determined. The response rate from the SHAs are also given in Table 2, although these data could not be calculated for all SHAs, as the destination of the questionnaire in these SHAs was not made available to the researcher.

Table 2. Number of Participants per Strategic Health Authority

SHA	**Number of Respondents**	**Response Rate (%)**
North West	1	ND
North East	6	ND
London	3	33.3
South Central	4	21.1
West Midlands	4	ND
East Midlands	2	ND
South West	5	ND
East of England	3	7.70
Yorkshire and the Humber	2	ND
South East Coast	5	18.5
Wales	4	18.2
Scotland	4	44.4
Unknown	1	ND
Total Number of Responses	44	ND

ND = not determined

2.4.3 Questionnaire Analysis and Interpretation

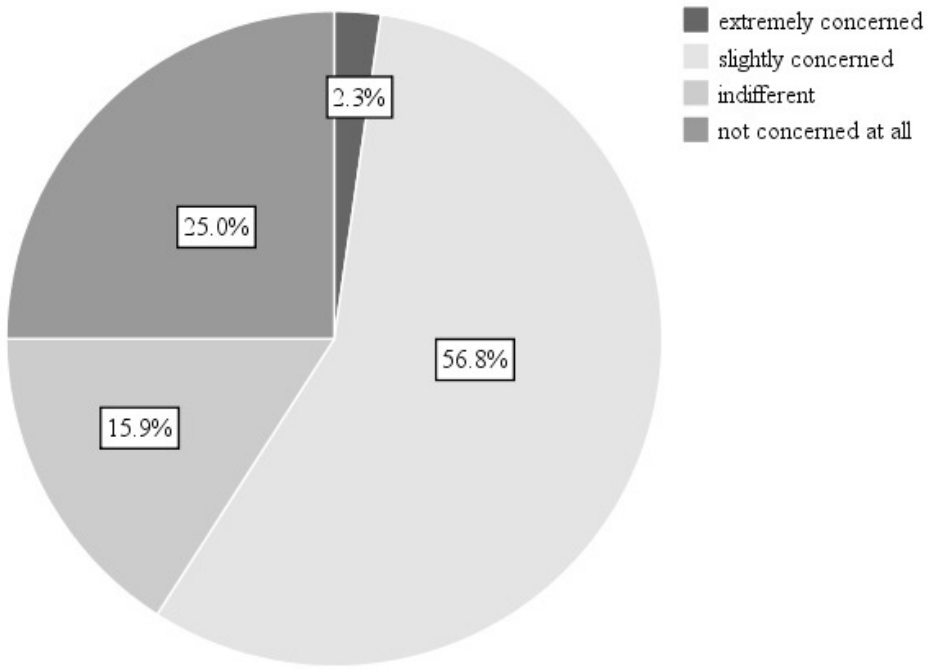
Isolators were used for the compounding of cytotoxic drugs by 44 ASUs (100%). None of the ASUs used BSCs or any other areas for this type of work. Of these 44 isolators, 95.5% operated under negative-pressure and of this percentage,

40.5% were ducted internally and 59.5% were ducted externally. Only 4.5% of the isolators used, operated under positive-pressure, all of which were ducted externally.

2.4.3.1 Concerns Regarding Cytotoxic Contamination

In response to concerns regarding cytotoxic contamination, the personal view of only one (2.3%) participant was that they were 'extremely concerned', 56.8% were 'slightly concerned,' 15.9% were 'indifferent' and 25.0% were 'not concerned at all.' The results are represented in a pie chart; see Figure 24 below. Each portion represents the amount of concern.

Figure 24. Pie Chart Showing Concerns Regarding Cytotoxic Contamination in ASUs



2.4.3.2 Decontamination Procedures Used

After the use of isolators for the compounding of cytotoxic drugs, 6.8% of ASUs applied a cleaning procedure only, 4.6% applied a disinfection procedure only, and 88.6% applied both a cleaning and a disinfection procedure.

2.4.3.3 Cleaning and Disinfection Procedure

Table 3 below, shows the frequency of usage of water, alcohol, detergents and liquid biocides for cleaning and disinfecting the isolator after the compounding of cytotoxic drugs.

Table 3. Frequency of Product Usage for Cleaning and Disinfecting the Isolator

	Number of ASUs	
Product	**Cleaning**	**Disinfection**
Water (all qualities)	8	0
Alcohol (all qualities)	39	20
Detergents		
Neutral	4	2
Amphoteric	3	1
Liquid Biocides		
QAC/biguanide	6	7
QAC/CD	4	11
Hydrogen peroxide	1	4
QAC	1	13
CD	2	5

QAC = quaternary ammonium compounds
CD = stabilised chlorine dioxide

Cleaning

Water was used for cleaning the isolator only by 8 ASUs (18.2%). WFI and water for irrigation were the water qualities used by 7 ASUs. Neither distilled nor

deionised water was used for cleaning, one unit did use an 'other water quality' once a day, five days a week, but they did not specify the quality. Water was used by 6 ASUs daily, and by 2 weekly.

Alcohol was used by 39 ASUs (88.6%) for cleaning the isolator. All used it on a daily basis. Denatured ethanol, 70% v/v (IMS) in a spray form (76.9%) was the main choice of alcohol and application method, compared to impregnated wipes (35.8%), some used both. Isopropyl alcohol (IPA) spray was used in only 1 ASU for cleaning the isolator, twice daily. IPA wipes were used for cleaning the isolator in 6 ASUs. On a daily basis the isolator was cleaned once (13 ASUs), twice (9 ASUs), three times (2 ASUs), four times (3 ASUs), five times (2 ASUs) and more than 10 times by one ASU with IMS spray. 'Other alcohol' was used by one ASU; they did not specify the type.

Detergents, including liquid biocides were used for cleaning by 21 ASUs (47.7%). The detergents used for cleaning were either neutral detergent or amphoteric surfactant. Neutral detergent was used by more ASUs (4 compared to 3) than amphoteric surfactant. The liquid biocides used for cleaning contained QACs, QACs/biguanide, QACs/stabilised chlorine dioxide, and hydrogen peroxide. The interval of using these products were once a day, once a week, three times week, once a month, twice a month and twice a year. The liquid biocide blends of QACs /biguanide (6 ASUs) and QACs/stabilised chlorine dioxide (4 ASUs) were also used commonly.

Disinfection

Alcohol, and the same detergents and liquid biocides, which were used for cleaning, were also used for disinfection. Alcohol was used by 20 ASUs (45.5%) and

was used commonly for disinfection on a daily basis, sometimes more than 10 times a day.

Detergents were used by 3 (6.8%) and liquid biocides by 40 ASUs (90.9%) for disinfection of the isolator. Again, neutral detergent and amphoteric surfactant were the only two detergents mentioned and neutral detergent was the used most commonly (2 compared to 1 ASU). QACs were the most common liquid biocide, followed by QACs and stabilised chlorine dioxide blends, used by 13 and 11 ASUs, respectively. QACs and biguanide blends were also popular; these had not been listed on the questionnaire but were used by 7 ASUs. Stabilised chlorine dioxide and QACs were used daily, but no more than twice. QACs, and QACs and chlorine dioxide blends were more likely to be used as disinfectants on a monthly or yearly basis.

Sodium hypochlorite was only used by 1 ASU, once a month. Stabilised glutaraldehyde and chlorhexidine were not used to disinfect the isolator by any of the ASUs.

The application method for disinfection of the isolator was only completed by 17 of the 30 participants who used detergents/liquid biocides. Using a spray and a dry wipe was the most common method of detergent/liquid biocide application for disinfection. Sprays only, and liquid with dry wipes, were used each by 1 of the ASUs. They were used for applying alcohol, and another liquid biocide, the name of which was not mentioned. Impregnated wipes were used by 6 of the applications, and spray with dry wipes by 16 out of 24 of the respondents.

Only 4 ASUs (9.1%) used fumigation/gassing for disinfection of the isolator. Peracetic acid was used twice a month in 1 ASU, and formaldehyde was used in 3 ASUs, once weekly, once yearly, and twice yearly. The process that was described

by one participant was to boil off 50% formaldehyde and 50% water in a heated mantle placed within the isolator. Hydrogen peroxide and ozone gas were not used for fumigation/gassing to disinfect any of the isolators.

2.4.3.4 Change in Decontamination Procedure Depending on the Drug Manipulated

Of the different decontamination procedures described by the participants, 39 out of 44 (86.6%) of these procedures did not differ according to which drugs had been manipulated in the isolator. One reported that the procedure would change if there was the occurrence of a cytotoxic antibiotic spillage.

A change in these procedures, whether they involved cleaning only, disinfection only, or both combined, occurred in 5 ASUs (11.4%), according to which drugs had been compounded in the isolator. The reason was due to the preparation of MABs *i.e.* Herceptin and Mylotarg. The procedure was specified for 2 ASUs. One ASUS, after the preparation of MABs would clean using a biocide followed by IMS, the other, would clean with IMS spray followed by IPA wipes. It was mentioned by two participants that they have the facilities to use separate isolators, one for the preparation of cytotoxics and another for the preparation of MABs. In addition, they had extra cleaning procedures in place in the event of only one isolator being operational, which in one case was a regular occurrence. None of the participants reported that they changed their cleaning procedure according to the type, or class, or chemistry of the cytotoxic drugs compounded.

2.4.3.5 Purpose of the Decontamination Procedure

All, except one participant (2.3%) replied to the question, which asked the purpose of the decontamination procedure of the isolator. A small percentage, 2.3% chose that it was ‘to remove cytotoxic contamination’ or, 9.1% ‘to disinfect/sterilise

the area'. The main responses were that the purpose of their decontamination procedure had a dual aim *i.e.* 36.4% said that it was 'to maintain a sterile environment and remove some cytotoxic contamination'; 50.0% said that it was 'to maintain a sterile environment and remove all cytotoxic contamination'.

2.4.3.6 How the Decontamination Procedure was Devised

Information regarding the source of the decontamination procedure was provided by 42 of the 44 participants. This question was not responded to by 2 participants. Of the responses, 54.5% had relied on, and specified, more than one source. The main sources of national guidelines were Quality Assurance of Aseptic Preparation Services,[141] The Cytotoxics Handbook,[24] Pharmaceutical Isolators,[142] Aseptic Services Specialist Interest Group (ASSIG) guidelines,[143] Rules and Guidance for Pharmaceutical Manufacturers and Distributors (MCA).[135] International guidelines had also been used to devise the procedures; however, the participants provided no reference to any of the sources of these international guidelines. Influence from local ASUs, the regional quality control laboratory, and experience from working in other ASUs were also sources. Other sources included onsite quality control advice, own collective experience, and advice provided from quality assurance, as a result of audits. Advice from manufacturers was also a contributor but only one company was mentioned.

2.4.3.7 Effectiveness of the Decontamination Procedure

Few of the ASUs (17) had measured cytotoxic contamination. Out of these, 7 had found cytotoxic contamination in one of the areas listed in the questionnaire. It was commented on by some, that the facilities for measuring levels of cytotoxic drugs were not available locally, and that only a limited number of cytotoxics could

be tested for. It was also mentioned, referring to the methods, that they are *'complex procedures using expensive equipment and accessibility to them are limited.'*

Overall, cytotoxic contamination had been measured in all of the areas listed, except for airborne levels. Table 4 below, expresses the number (and percentage) of ASUs which had measured for cytotoxic contamination in the areas specified in the questionnaire, and from those which had measured, the number (and percentage) which had found cytotoxic contamination. The frequency of measurement and the levels of contamination found were not specified by the ASUs.

Table 4. Number of ASUs and Areas where Cytotoxic Contamination has been Measured

Area	**Number (%) of ASUs**	
	Contamination Measured	**Contamination Found**
Vials	7 (15.9%)	2 (28.6%)
Dispensed product	3 (6.8%)	0
Surfaces inside the isolator		
before decontamination	10 (22.7%)	4 (40.0%)
after decontamination	9 (20.5%)	2 (22.2%)
Surfaces outside the isolator		
before decontamination	10 (22.7%)	2 (20.0%)
after decontamination	5 (11.4%)	1 (20.0%)
Airborne	0	0
Gloves used in the isolator	3 (6.8%)	1 (33.3%)
Gloves used outside the isolator	1 (2.3%)	0

The highest number of cases (4 out of 10) when contamination had been found was on surfaces inside the isolator. After the decontamination procedure had

been carried out, contamination on surfaces inside the isolator was still present in 2 out of 9 cases (1 ASU did not measure for contamination after decontaminating the isolator). In 1 out of 3 cases, the gloves used inside the isolator tested positive for contamination, although it had only been measured for in 3 ASUs. Contamination had also been found on vials (2 out of 7 cases). One ASU reported heavy contaminated of DOX powder observed visibly on the external surface of vials, as supplied by the manufacturer. The other contaminating drugs found on the external surface of vials were not specified by the ASU. Measurements taken for cytotoxic contamination of the dispensed product and the gloves used outside the isolator had not tested positive.

Included in other areas, one ASU had measured for cytotoxic contamination in the preparation room as part of an awareness exercise. Low levels were found on the telephone and door handles. As a result, the cleaning procedure was reviewed and after repeating the exercise, no contamination/low levels were found. The levels of contamination found were not provided by any of the ASUs, neither was the LoD of the analytical method used.

The same facility had also measured for cytotoxic contamination on surfaces inside the isolator, interestingly none had been found. Other ASUs had measured for contamination on pens, scissors, bench tops, re-usable work trays, transit bags of the finished product, work surfaces outside the clean-room, fridges, and the cytotoxic floor, where none hand been found.

One facility in particular included a risk assessment form with their response. The assessment had been carried out after finding cytotoxic contamination present in the isolators, and on floors and benches in the checking areas of the isolator room. Repeat testing after the introduction of the control measures listed, in the risk

assessment, had eliminated contamination in areas outside of the isolator and significantly reduced levels in the isolator.

In 4 of the ASUs, which had the facilities to test the effectiveness of the decontamination procedure applied to the isolator, 2 of the procedures had been effective in removing cytotoxic contamination. One procedure was to clean with WFI (once a day), and IMS spray and IPA wipes (both 10 times a day), followed by disinfection with alcohol spray and dry wipes more than 10 times daily, QACs/stabilised chlorine dioxide impregnated wipes (both once a day), and fumigation with formaldehyde once a week. The other effective procedure was to clean with water for irrigation, IMS spray and wipes, neutral detergent and QACs/biguanide daily followed by disinfection with alcohol and QACs (frequency not specified).

2.4.3.8 Concerns and Problems Encountered when Manipulating Cytotoxic Drugs

This open question was commented on by 19 (43.2%) of the participants. Issues of concern regarding the supply of contaminated vials to the pharmacy were mentioned on 3 occasions. One unit had received a visibly very heavily contaminated vial of DOX powder, which consequently needed extra cleaning. This contamination was visible to the naked eye, but the concern was that contamination on a vial of cisplatin would not be visible. The comment was made that the responsibility should belong to the manufacturers to provide contamination free vials and packaging, and it should not rely so heavily on the individual ASUs to routinely *"have to wipe each vial 5 times."*

The lack of national guidelines, no data on safe low *i.e.* picogram levels of exposure, concern regarding long-term exposure and the lack of biological tests were mentioned on several occasions. In particular, one participant expressed their

difficultly in reassuring their staff of the safety of long-term exposure. Another participant, further questioned "if there was a limit from extensive testing what relevance would the results have, how would it relate to personnel exposure, what levels of detection and contamination would be considered acceptable, what additional means could then be put in place?"

One participant explained they are aware of the research carried out in this area but that they *"have difficulty with projects based on measuring cytotoxic contamination levels, given that there is no guidance on what is an acceptable level of contamination. Clearly, the approach can only establish a relative reduction in contamination, to the lowest level of detection i.e. like radiation; I do not believe that a safe level of contamination can be defined. Therefore, the research objectives must be to compare different cleaning procedures and establish which are the most effective that can be recommended to users."* Another participant suggested that it would be interesting to see some *"best practice"* guidelines for decontamination of equipment following the handling of cytotoxics.

The opinions of 3 participants were that they had no issues and that they felt that the risk to the operators was low. One quoted *"operators are suitably trained in the safe manipulation of cytotoxic drugs with the correct equipment to reduce the risk of aerosol formation and contamination; I feel the risk is low, given that all these manipulations are carried out in a contained environment."*

One participant only, mentioned that they felt cytotoxics were being handled in an inappropriate manner; the reason was attributed to staff shortages and a constant increase in workload.

2.5 Discussion

The questionnaire was sent out to explore the procedures which are currently used to decontaminate areas where cytotoxic drugs are compounded. It was sent to pharmacists who manage ASUs in NHS hospitals in England, Scotland and Wales. These hospitals are regulated by the Department of Health and are subjected to external audit by the regional Quality Assurance Pharmacist every year, in accordance with EL (97) 57, against common standards set by the NHS Pharmaceutical Quality Control Committee.[141] Private hospitals were excluded from this study, as they are not regulated or subject to regular external audit. This results in varying standards of aseptic preparation practice between private hospitals, which may not always be equivalent to the level of NHS hospitals.

The number of responses received *i.e.* the number of participants was 44. This was a lower response than anticipated compared to a possible 181 responses. Responses were received from hospitals in all twelve SHAs. The highest number of responses was from the North East (6 responses) and the South West (5 responses). There was only one response from the North West. The response rate could not accurately be determined for all areas, *i.e.* The North West and East, West and East Midlands, The South West and Yorkshire and the Humber, as it was not known exactly how many people it was sent to in these SHAs. When sending out the questionnaire it was requested that the email recipients sent to were copied to the researcher, but this was not done for the email recipients in these SHAs. Of those for which the response rate could be calculated, Scotland and London gave the highest response rates, 44.4% and 33.3%, respectively - see Table 2 (page 67). Although national participation in the questionnaire was observed, the results of the questionnaire could not be generalised to be representative of current practice in the

UK as this would be dependent on obtaining a complete and accurate list of the population and a higher and accurate response rate.

Follow-up reminders to non-responders preceded by two reminders enclosing a replacement questionnaire may have facilitated response rates.[144] In this present study, if follow-up reminders enclosing the questionnaire had been sent to non-responders, which was the study design (Section 2.3.6), the response rate might have been improved. However, the participants of the questionnaire who responded *via* the paper method and chose confidentiality as to the name of the hospital could not be identified. This meant that their identity could not be communicated to the chairs and secretaries of the National Pharmaceutical Production Committee, who distributed the questionnaire, to remove them from the follow-up list. It may have been conceived, by those that had already participated, to be a nuisance to receive reminders for a questionnaire that they had already completed. Therefore, the decision was taken not to follow-up to attempt to enlist non-responders; the trade-off being a low response rate.

On reflection, a higher response rate may have been achieved in this study if control of the distribution of the questionnaire had remained with the researcher. This was not the case, and the advised method of distribution was to use a Specialist Pharmacy Interest Group which operates a communication network through its members comprising a representative from each SHA. The researcher could not be provided with a list of the contact details of the members of these groups, as it was the policy of these specialist groups to protect the identity of its members. Therefore, reliance was on the chairs and secretaries of these groups to distribute the questionnaire. Although there is no up-to-date list of contact details for pharmacy managers of ASUs available, a list of contacts could have been generated using the

Chemist and Druggist Directory[139] or each individual NHS website to telephone each Hospital Trust and ask for the contact details of the relevant ASU pharmacist. A higher response rate may also have been observed using an alternative data collection method. Face to face interviews are recommended to achieve the best response rate.[144] However, interviews are time-consuming and costly, and would have been impractical in this case due to the large geographical area. They are also suited for more complex answers and the collection of a large amount of data. Computer-assisted telephone interviewing is a rapid method of collecting data[145] and may give a higher response rate than sending out a questionnaire.[144] Using the self-generated telephone list of contacts, the pharmacist could have been invited to participate in the study via telephone and a convenient time to conduct the computer-assisted telephone interview arranged. This would have enabled direct control over the recruitment of participants and a quicker and probably higher response rate would have been observed.

The number of participants was adequate to explore this area of research and carry out descriptive statistical analysis. Almost twice as many of the responses received to the questionnaire were electronic compared to paper versions, simplifying data handling procedures. The rate of electronic response to this questionnaire (62.8%) was comparable to the rate of acceptability to electronic responses (57%), which was observed to be the preferred method of response in a study comparing electronic with the conventional paper questionnaire.[138]

It is very concerning that nearly half of the negative-pressure isolators used in ASUs (40.5%) are ducted internally *i.e.* the air is re-circulated (see Section 2.4.3). It is recommended by NIOSH that ducting from the isolator should be to the outside

environment, unless the drugs used, do not volatise during manipulation or after capture by the HEPA filter.[4] It is also concerning that a quarter of the participants were 'not concerned at all' about cytotoxic contamination in the isolator - see Figure 24 (page 68). The reason may be that it is not the pharmacy managers' themselves who manipulate cytotoxic drugs, but pharmacy technicians and assistants. It would be interesting to learn of the response to this question from the technicians and assistants who actually carry out cytotoxic manipulations. It is somewhat reassuring that the largest number of participants (56.8%) were 'slightly concerned'. Only 2.3% (1 participant) was 'extremely concerned'.

A large percentage of ASUs (88.6%), apply a cleaning and a disinfection procedure for the decontamination of the isolator, after its use for the compounding of cytotoxic drugs. Water (18.2%), alcohol (88.6%), and detergent/liquid biocides (47.7%) were used for cleaning the isolator – see Table 3 (page 69). The water used was WFI or water for irrigation, which is the high quality water recommended for cleaning isolators,[110] although water was not used commonly for cleaning this area. As most cytotoxic drugs are water-soluble (usually salts), water would be a suitable solvent for the removal of cytotoxic contamination, more so that alcohol, which is a disinfectant; it is not a good cleaner. Water should also be used after cleaning with detergent to remove any residue.[110]

Alcohol was used less for disinfection (45.5%) compared to cleaning (88.6%) of the isolator. IMS spray with dry wipes was the alcohol and application method of choice for disinfection of the isolator. IMS, in a spray form, was used twice as much as IMS-impregnated wipes. However, spray cleaners run the risk of damaging the HEPA filter, and there may be a hazard, if vapours build up and there is inadequate

ventilation.[49] The frequency of cleaning with IMS spray was generally once or twice a day.

Gassing/fumigation were only used by 4 (9.1%) ASUs for disinfection of the isolator. There is an increasing need for disinfection in hospitals due to the emergence of resistant pathogens, especially where pharmaceuticals are aseptically prepared. A study carried out to assess biological disinfectant usage in hospitals pharmacy ASUs, reported that alcohol was used most commonly by 60.7% to disinfect the surfaces of isolators,[146] a higher percentage than demonstrated in this study.

Neutral detergent, QACs and QACs combined with other compounds *i.e.* biguanide and stabilised chlorine dioxide, were the common detergents/liquid biocides of choice. Of the two detergents mentioned for cleaning and disinfection neutral detergent was used most commonly. QACs/biguanide blends were used most commonly for cleaning; QACs/stabilised chlorine dioxide blends were also used frequently. QACs were used most commonly for disinfection closely followed by QACs/stabilised chlorine dioxide blends. Combining the two processes, QACs/stabilised chlorine blends were the liquid biocides used most commonly, and were applied as a spray and wiped using a dry wipe (Section 2.4.3.3). Interestingly, sodium hypochlorite, which may be sporicidal, was not a common choice for decontamination by the ASUs. This may be because there has been a reported increase in the use of other halogen-based agents which are also sporicidal, and QACs containing added surfactants which may improve cleaning properties.[146]

For disinfection, liquid agents were the choice over gassing/fumigation methods (Section 2.4.3.3 - disinfection). The reason may be that they are easier and quicker to use and specialised equipment is not required. The gassing or fumigation

of an isolator, if performed correctly, can ensure access of otherwise inaccessible areas. They are not sterilisation processes but are used to reduce the bio-burden in the isolator to a defined level. Fumigation has been used traditionally for many years and describes gassing when formaldehyde is the sanitisation agent. Formaldehyde was still being used in 3 ASUs, once a week in one ASU, however there are serious concerns about its toxicity and it may be potentially carcinogenic.[110;147] This may be another reason why liquid biocide/detergents were preferred to fumigation/gassing methods. Peracetic acid was used only by one ASU, supporting the literature that reports its limited use.[110] It is heated to produce a vapour, which is harmful but decomposes to acetic acid and water, both of low toxicity.

The view of 50.0% of the participants, who specified the decontamination procedure, was that it was 'to maintain a sterile environment and remove all cytotoxic contamination' (Section 2.4.3.5). No one specified that it differed according to the type of cytotoxic drug manipulated. It is apparent from the decontamination procedure described, that the main aim concerns a sterile environment, but there appears to be little or no consideration regarding cytotoxic chemical removal.

Those that are aware of the risks, have only limited information resources, with reliance being heavily on national guidelines from a few select books available, and advice from quality control staff. The literature is lacking regarding choice of disinfection agents for cytotoxic chemical decontamination. Although measurements of cytotoxic contamination are well published in the literature, the levels that are acceptable have not been defined. This is not surprising since levels causing human toxicity are also not defined.

The ASUs which had measured for cytotoxic contamination, had found it on vials, gloves used in the isolator, surfaces inside the isolator before and after decontamination, and surfaces outside the isolator – see Table 4 (page 74). Information regarding the contaminating drug or the levels found were not provided. The findings of contamination in one unit had resulted in improved protective measures, which were effective. It may have been informative to have asked in the questionnaire for the frequency of measurement in each of the areas, the levels of contamination found, the contaminating drug and the LoQ of the analytical method applied. In any event, there appears to have been little thought given to the design of monitoring schedules for cytotoxic contamination. For example, isolator gloves would be considered a major area of contamination, yet only 3 out of 17 ASUs who monitored contamination included isolator gloves in their schedule.

It was apparent that some ASUs have had to introduce and accommodate for the production of MABs (Section 2.4.3.4). Those that mentioned that they were preparing MABs were aware of the possibility of cross-contamination. A separate isolator for the production of MABs was used where available. Decontamination procedures were in place with the aim of preventing cross-contamination, if prepared in the same isolator as cytotoxic drugs, or after preparation in a separate isolator. However, there was little evidence that these procedures were routinely validated, if they were validated at all.

The questionnaire was designed to ascertain the decontamination procedures, which are being carried out in NHS hospitals, which have ASUs. There is no written standard for these procedures, against which to evaluate the responses. Concerns about cytotoxic contamination ranged from extreme to no concern, and would reflect current safety of practice.

2.6 Conclusion

The use of isolators and the preference towards negative-pressure isolators in NHS hospital pharmacies is in agreement with the literature.[75] The most prevalent practice in hospital pharmacies is to decontaminate an isolator after the manipulation of cytotoxic drugs according to a protocol, which covers cleaning and disinfection. To clean the isolator IMS spray would be used, IMS wipes are also in use, as are IPA wipes, and WFI or water for irrigation. Alcohol would also be used for disinfection. In addition, detergents/liquid biocides are used for both cleaning and disinfection. Neutral detergent, and the liquid biocides *i.e.* QACs, QACS blends with stabilised chlorine dioxide or biguanide are common. These detergents/liquid biocides are currently the choice of use over fumigation/gassing methods for disinfection.

This study indicates that the understanding of mechanisms of cleaning and decontamination by ASU managers is currently very low. The lack of structured, robust validation and monitoring procedures into the majority of ASUs is a major cause of concern, particularly with the increasing use of biological agents such as MABs.

3. Development and Validation of Sampling/Quantification Methods

3.1 Introduction

To assess the risk to personnel, cytotoxic marker drugs are selected as the most significant analytes. The most toxic, according to the IARC, the drugs used most extensively and the availability of suitable sensitive analytical methods are considered when selecting marker drugs.[8] It was necessary to develop and validate reliable analytical methods to identify and quantify the marker drugs after the experimental phases in Chapters 4 and 5 of this thesis.

Method 1 was developed and validated to quantify cytotoxic surface contamination after the decontamination procedures carried out in Chapter 4. 5-FU, CP, DOX and EPI were the cytotoxic marker drugs used. The decontamination procedures were:

Phase I - physical removal of cytotoxic contamination from a surface by wiping with a) detergents of different pH and b) detergents used commonly in hospital practice,

Phase IIi - degradation of cytotoxic contamination by exposure to the same detergents applied in Phase I,

Phase IIii - degradation by oxidation of cytotoxic contamination on a surface from exposure to vaporised hydrogen peroxide (VHP®).

A recovery method was developed and validated to remove the drug from a surface into a desorbing solution after wiping with detergents (Phase I) and exposure to VHP® (Phase IIii). HPLC methods were validated to quantify the amount of drug recovered from the surface in Phase I and IIii, and quantify the amount of drug degraded after exposure to the detergents (Phase IIi).

Method 2 was developed and validated to recover multi-drug surface contamination into a desorbing solution for analysis by HPLC. EPI, MTX and CP were the drugs compounded in an isolator in a two-arm cross-cohort study, as investigated in Chapter 5. Two systems for the compounding of cytotoxic drug injections/infusions were compared; the traditional open-system and use of a closed-system (PhaSeal®) device for fluid-transfer. The multi-drug contamination produced was recovered from surfaces inside the isolator, immediate surfaces outside the isolator and syringe batches prepared in the isolator.

Recovery Methods – Wipe sampling and Immersion

Surface contamination is measured typically by removing the contaminants from the surface to an absorbing material and/or solvent and subsequent desorption of the drug contamination from that material into another solvent (desorbing solution). Wipe sampling and immersion were used to remove and recover the drug from various surfaces in this study.

Wipe sampling is a valuable tool for measuring the residual contaminants in the workplace and to assess the effectiveness of personal protective equipment and decontamination procedures.[148] Many studies have applied this method to evaluate surfaces for the presence of cytotoxic contamination.[20;28;29;42;58;66;108;109]

Immersion is a method typically used for measuring cytotoxic contamination on disposable surfaces of a more complex topology, which are difficult to wipe *i.e.* gloves.[28;29] The surface is placed into a desorbing solution which the drug will have affinity for, and mechanical methods *e.g.* mixing, vortexing[60;149;150] shaking[148] and centrifugation[60;149;150] have been used to extract contamination from a surface into a desorbing solution.

High-Performance Liquid-Chromatography

HPLC is used commonly for the quantification of pharmaceutical products. It provides accurate, precise, selective and robust methods for analysis and is the industry standard for this purpose.[104] The cytotoxic marker drugs investigated were recovered and diluted in aqueous solution. Reverse-phase chromatography involves a non-polar stationary phase and a relatively polar mobile phase. Mobile phases are usually aqueous-organic solvent mixtures that may be buffered. Mixtures are separated by subtle differences in hydrophobicity; the more hydrophobic the analyte the longer it is retained on a reverse-phase column.

The light absorption of a drug molecule is due to the particular combination of auxochromes and chromophores present in its structure.[104] UV detection is the most commonly used method of detection for pharmaceutical compounds by HPLC,[104] although fluorescence detection is also used and offers greater sensitivity. Fluorescence is associated with an extended chromophore/auxochrome and a rigid structure.[104] When the fluorescent spectrum of a molecule is scanned, two maxima are observed, the first maxima is due to the scatter of excitation radiation and the second maxima is due to fluorescence emission.

Method Validation

Methods must be validated to ensure integrity, and demonstrate that they are reliable and reproducible. Validation must be carried out prior to routine use of the method, and involves documenting the use of specific tests to provide evidence that the method is suitable for application, and does what it is intended to do.[151] All the variables of the method should be considered, from sample collection and storage, sample preparation, the sample diluent (desorbing solution), chromatographic

separation detection and data evaluation.[151] Validation of the analytical method may include determining selectivity, precision, accuracy, recovery, LoQ and LoD, and stability under study conditions.[152]

3.2 Objectives

3.2.1 Method 1

The objectives of Method 1 were to:

i. identify suitable cytotoxic marker drugs which are likely to degrade by different mechanisms,
ii. identify a suitable test surface on which to expose the drugs,
iii. identify a suitable desorbing solution for each drug,
iv. develop a recovery method with high and reproducible recovery for the quantification of all drugs.

3.2.2 Method 2

The objectives of Method 2 were to:

i. identify suitable cytotoxic marker drugs for compounding in an isolator,
ii. identify surfaces inside and outside the isolator from which to recover cytotoxic contamination of the drugs,
iii. identify a suitable wipe material and method to remove surface contamination of these drugs,
iv. identify a desorbing solution which the drugs will have affinity for to recover contamination of these drugs from the wipe or surface,
v. identify a recovery method to remove the drug contamination from the wipe or surface into the desorbing solution,

vi. develop and validate sensitive analytical methods to determine contamination of these drugs at low concentrations.

3.3 Materials

Cytotoxic Drugs

5-Fluorouracil 25 mg mL^{-1} (lot N022669), Doxorubicin Hydrochloride 50 mg for Injection (lot N062326) and Methotrexate 25 mg mL^{-1} Injection (lot R044426) were obtained from Mayne Pharma Plc, Leamington Spa, UK. Cyclophosphamide 500 mg Powder for Injection (lot 3K114A) was obtained from Pzifer Ltd, Kent, UK. Pharmorubicin Solution for Injection 2.0 mg mL^{-1} (lot BD67A) was obtained from Pharmacia and Upjohn Ltd, Sandwich, UK.

Diluents

Normal saline 0.9% (lot MK7209 04) and sterile water for injections (lot 04C27B25) were obtained from Baxter, Newbury, UK.

Chemicals

Acetonitrile (lots 0440085, 0553508, 0570049, 0572514, 0584171 and 0607839), disodium hydrogen orthophosphate (lot 0307261), sodium dihydrogen orthophosphate (lot 0389539), methanol (lots 01417856, 0428369, 0432580 and 0583764), potassium dihydrogen orthophosphate (lot 0398776) and sodium hydroxide (lot 0422611), were purchased from Fisher Scientific, Leics, UK.

Ammonium sulfate (lot A201501), hydrochloric acid (1 M) (lot OC333385), perchloric acid (lot B23634917), sodium chloride (lot MK720904) and sulfuric acid (lot K29485908) were purchased from BDH, Poole, UK.

Potassium permanganate (lot D2.703) was obtained from Sigma Aldrich Co Ltd, Dorset, UK, and hydrogen peroxide (lot BN24WD) was obtained from Thornton and Ross, Huddersfield, UK.

All chemicals and reagents used for HPLC were of analytical or HPLC grade.

Equipment

The HPLC system comprised an LDC Analytical isocratic constaMetric 3200 pump (serial no. 048086) from Thermo Separation Products, Stoke on Trent, UK, an 851-AS autosampler (serial no. C6701589) from Jasco, Great Dunlow, UK, coupled with a variable wavelength UV detector 785A (serial no. 9106344) from Applied Biosystems, Warrington, UK, or a fluorescent detector FP2020 (serial no. B035760869) from Jasco, Great Dunlow, UK. Data analysis was performed using Prime software, Version 4.2.0 from HPLC Technology, Herts, UK.

Stainless steel column, 150 × 4.6 mm packed with Columbus C_{18}, 5 µm particle size (serial no. 414370) was purchased from Phenomenex, Cheshire, UK. Stainless steel column 150 × 4.6 mm packed with Techsphere C_{18}, 5 µm particle size (serial no. 332-04, 789-04); stainless steel column 250 × 4.6 mm, packed with Techsphere C_{18}, 5 µm particle size (serial no. 590-04, 05031778.1) and stainless steel column packed with Techsphere CN, 250 × 4.6 mm, 5 µm particle size (serial no. 325-04, 05021707.1, 05040838.2 and 605063021.1) were purchased from HPLC Technology, Herts, UK.

The glass electrode and pH meter 302 (serial no. 336888) were from Hanna Instruments, Bedfordshire, UK. The AE163 balance (serial no. B68994) was from Mettler Toledo, Leicester, UK. The BRi4 centrifuge (serial no. 30002493) was from Jouan, Winchester, UK and the Class II biological safety cabinet (serial no. MC4607-

1) was from Medical Air Technology, Oldham, UK. The UV spectrophotometer CE5501 (serial no. 84203) & graphic plotter CE5501 (serial no. 85226) was made by Cecil Elegant Technology, Cambridge, UK. The water bath with stirrer, model NE4D (serial no. 46223) and the vortex FB65000 (serial no. 46998) were made by Nickel Electro Ltd, Weston-super-Mare, UK. The Certomat M orbital shaker (serial no. 886008/4) was made by B.Braun, Sheffield, UK. Variable volume Gilson pipettes, P2-P5000, (serial no. X53584G, T55103N, T62823, N55454L, T53730K and T60889J) were purchased from Anachem, Bedfordshire, UK, and the vacuum and filtration unit were purchased from Millipore, Watford, UK. The Proline PL1218W freezer (serial no. 300516015020) was purchased from Comet Group Plc, Bath, UK. The Swingwave autoclave Type: S.F.T-lab was from Ilford, Essex, UK.

Consumables

Mixed cellulose-ester membrane filters, 0.45 µm pore size, 47 mm diameter (lot R4HN57575) were obtained from Millipore, Watford, UK. Polypropylene centrifuge tubes (15 mL) and polypropylene containers (250 mL) with screw caps were from Sarstedt, Leics, UK. Polypropylene Plastipak syringes (5 mL) were obtained from Beckton and Dickinson, Oxford, UK. The HPLC autosampler vials and caps were obtained from HPLC Technology, Herts, UK,

Cliniwipes IPA 200 and Spiriclens sterile session wipe (lot 14110) were obtained from Adams Healthcare, Leeds, UK. Klerwipe™ 70/30 sterile alcohol-impregnated wipes were from Shield Medicare, Farnham. Stericlean® Flowrap (lot W016603) and Stericlean® prep pads (lot 529179) were from Helapet Ltd, Bedfordshire, UK.

To achieve the objectives described in Section 3.2, two principal methods were developed.

Method 1 to quantify the amount of 5-FU, CP, DOX and EPI remaining or degraded after the decontamination procedures described in Chapter 4.

Method 2 to quantify cytotoxic surface contamination of EPI, MTX and CP after the compounding of cytotoxic drugs in an isolator, as described in Chapter 5.

3.4 Method 1 - Development

3.4.1 Selection of Drug

5-FU, CP, DOX and EPI were the cytotoxic drugs used frequently in the treatment of cancer, which were selected as marker drugs for this study. 5-FU, an antimetabolite - see Figure 8 (page 8); CP, an alkylating agent - see Figure 2 (page 4); and DOX, an anthracycline antibiotic - see Figure 13 (page 12), are structurally diverse and are known to be sensitive to degradation by different mechanisms *i.e.* hydrolysis and oxidation.[5;24;68;128;153;154] CP is one of the most carcinogenic (Group 1) of the cytotoxic drugs according to the IARC.[9] 5-FU has been used as a marker drug[8;62;67;124] and methods are plentiful in the literature for the analysis of 5-FU and DOX.[8;40;44;62;65;124;154-156] EPI was chosen because of its increasing clinical use, and for its structural similarity with DOX, and hence a similar stability profile. DOX may be degraded by the decontamination procedures carried out in Chapter 4. It exhibits a stable pH range between 3 – 7,[128] and may be degraded by the detergents of different pH formulated outside this narrow range. It is also subject to oxidation[153] and may be degraded by the oxidising agent VHP®. EPI was investigated to ascertain if it may be degraded by the same conditions that may degrade DOX. If the same detergents or exposure conditions to VHP® degrade DOX and EPI, then they may be applied to

degrade cytotoxic contamination of other anthracyclines *i.e.* daunorubicin. This may allow a prediction of effective decontamination agents for chemical families of cytotoxic drugs where there is structural similarity.

3.4.2 Drug Dilution and Reconstitution

5-FU, CP, DOX and EPI were reconstituted or diluted in three diluents. Two of the diluents were common to all four drugs, and were the pharmaceutical diluents WFI and NS. The third diluent was the aqueous part of the mobile phase specific to the HPLC assay used to quantify the drug *i.e*.

5-FU: phosphate buffer (0.01 M) pH 7.0,

CP: ammonium sulfate buffer (0.01 M) pH 3.5,

DOX: sodium chloride solution (0.01 M) adjusted to pH 2.25 with perchloric acid,

EPI: phosphate buffer (0.01 M), pH 4.0.

5-FU (25 mg mL^{-1}) as received was diluted in three diluents to give a working concentration of 5.0 mg mL^{-1}.

CP (500 mg) as received was reconstituted in three diluents to give a working concentration of 20 mg mL^{-1}.

DOX (50 mg) as received was reconstituted in WFI to give a concentration of 2.0 mg/ml; this was further diluted in three diluents to give a working concentration of 1.0 mg mL^{-1}.

EPI (2.0 mg mL^{-1}) as received was diluted in three diluents to give a working concentration of 1.0 mg mL^{-1}.

3.4.3 Test Surface and Test Surface Coating

A chemically inert polypropylene surface,[157] which would not contribute towards chemical degradation, was selected on which to carry out the

decontamination procedures. This test surface was made by the transverse sectioning through the barrel of a 5.0 mL polypropylene syringe at 2.0 cm intervals. The resulting rings were cut in half giving rectangular surfaces with dimensions of 2.0 cm by 1.2 cm. The mass of the polypropylene pieces ranged from 0.22 to 0.26 g. The following were pipetted onto the concave side of the test surface to coat it:

5-FU ($5.0\ mg\ mL^{-1} \times 10\ \mu L$)

CP ($20\ mg\ mL^{-1} \times 20\ \mu L$)

DOX ($1.0\ mg\ mL^{-1} \times 10\ \mu L$)

EPI ($1.0\ mg\ mL^{-1} \times 20\ \mu L$)

All coated test surfaces were allowed to dry in a BSC. The time taken for the drug solution to dry was determined visually. The maximum amount of drying time required (until no solvent remained) was approximately 2 hrs.

3.4.4 Selection of Desorbing Solution

The aqueous part of the mobile phase specific for each drug assay (which was also the third diluent as described in Section 3.4.2) was selected as the desorbing solution. This would ensure that the drug had a high affinity for the desorbing solution and would be likely to desorb from the test surface into this solution. The desorbing solution should also be compatible with the HPLC method, minimising disturbance of dynamic equilibrium when the sample is injected, and ensuring total and immediate mixing with the mobile phase. Generally, the packaging material in reverse-phase HPLC columns is stable within the pH range of 2 to 8 therefore; the mobile phase and sample solvent should be formulated within this pH range.

The desorbing solution for CP was ammonium sulfate buffer (0.01 M, 1.0 mL) pH 3.5. The basic nitrogen on the heterocyclic ring of CP indicates that CP may be fully protonated and soluble at acidic pH. Phosphate buffer (0.01 M, 5.0 mL) pH

7.0 was the desorbing solution for 5-FU.[155] 5-FU is a weak base possessing two ionisable nitrogens with pKa values of 7.0 and 13.[104] At pH 7.0, these nitrogens would be 50% and >99.99% ionised, respectively, and 5-FU would be soluble. The desorbing solution for DOX was sodium chloride (0.01 M, 1.0 mL) adjusted to pH 2.25, and phosphate buffer (0.01 M, 1.0 mL) pH 4.0 was used for EPI. DOX and EPI are weak bases with a pKb of 8.2.[153] Therefore, they were both >99.9% ionised and soluble in the pH of the respective desorbing solution.

3.4.5 Immersion and Recovery Method of Drug from the Test Surface

Immersion methods were developed to remove and recover as much drug as possible from the test surface into the desorbing solution. Each test surface containing dried drug was placed into a centrifuge tube containing the respective desorbing solution specific for each drug (see Section 3.4.4) The tube was inverted 10 times, vortexed for 30 sec, followed by centrifugation for 5 min at 1500 × g (5ºC). An aliquot of the desorbing solution was transferred to autosampler vials for assay by HPLC.

3.4.6 HPLC Methods

HPLC methods in use in the Clinical Pharmaceutics Laboratory, Department of Pharmacy and Pharmacology, University of Bath, and published methods have been used to quantify 5-FU,[155] CP,[158] DOX,[159] and EPI.[160]

The aqueous portion of the mobile phase was prepared using Milli-Q grade water, and the pH was measured using a glass electrode and pH-meter. The mobile phase was degassed and filtered under negative-pressure through a 0.45 µm pore size, 47 mm diameter mixed cellulose-ester membrane filter prior to use. For each assay, elution was isocratic, the flow rate was 1 mL min^{-1} and a sample of 100 µL

was injected. Duplicate test injections were bracketed by injections of standard solutions. The chromatograms of all samples were processed using Prime software (Version 4.2.0).

5-FU was analysed using a 150 × 4.6 mm stainless steel column packed with Columbus C_{18} (5 µm particle size). The mobile phase was phosphate buffer (0.01 M) pH 7.0 with 5% methanol (v/v). Detection was by UV at 270 nm. The autosampler injection needle was flushed with 2 × 500 µl of methanol:water (50:50) v/v after each injection. The final concentration for assay was 10 µg mL^{-1}.

CP was analysed using a 250 × 4.6 mm stainless steel column packed with Techsphere CN (5 µm particle size). The mobile phase was ammonium sulfate buffer (0.005 M) pH 3.5 with 30% methanol (v/v). Detection was by UV at 210 nm. The injection needle of the autosampler was flushed with 2 × 500 µl of methanol:water (50:50) v/v after each injection. The final concentration for assay was 400 µg mL^{-1}.

DOX was analysed using a 150 × 4.6 mm stainless steel column packed with Techsphere C_{18} (5 µm particle size). The mobile phase was sodium chloride (0.01 M, adjusted to pH 2.25 with perchloric acid) with 40% acetonitrile (v/v). Detection was by UV at 254 nm. The injection needle of the autosampler was flushed with 2 × 500 µl of acetonitrile:water (50:50) v/v after each injection. The final concentration for assay was 10 µg mL^{-1}.

EPI was analysed using a 250 × 4.6 mm stainless steel column packed with Techsphere CN (5 µm particle size). The mobile phase was phosphate buffer (0.01 M)

pH 4.0 with 30% acetonitrile (v/v). Detection was by UV at 276 nm. The injection needle of the autosampler was flushed with 2 × 500 µl of acetonitrile:water (50:50) v/v after each injection. The final concentration for assay was 20 µg mL^{-1}.

Each column was flushed at the end of the sample run or at the end of the day. The flush solution was 10 column volumes of Milli-Q grade water, followed by either methanol or acetonitrile:water (50:50) v/v, which was also the storage solution.

3.5 Method 1 - Validation

The recovery method described in Section 3.4.5 was validated for all four drugs in three diluents. HPLC was used to quantify the recovered drug as described in Section 3.4.6. The following criteria were investigated to ensure a validated reliable method.

3.5.1 Recovery of Drug from the Test Surface

The desorption of drug from the test surface into the desorbing solution was measured against a standard (taken as 100%), which had not been subjected to recovery. Recovery of the analyte with acceptable precision and accuracy was considered necessary to obtain a validated method.

3.5.2 Retention of the Analyte

The retention time is the time measured between sample injection and the apex of the peak in the chromatogram.[161] It is characteristic of a compound but is not unique, and was used to provide a tentative identification of the compound within a peak window of +/-2%. 5-FU eluted at a retention time of 3.4 min, CP eluted at a retention time of 3.8 min, DOX eluted at a retention time of 3.8 min, and EPI eluted at a retention time of 4.3 min.

3.5.3 Precision, Accuracy and Recovery

Precision was measured as the coefficient of variation (CV). The CV (%) was calculated by dividing the standard deviation by the mean. Five samples were prepared and assayed on the same day (intra-day) and one sample was prepared and analysed daily on 5 separate days (inter-day) by HPLC. Precision around the mean was acceptable if the CV was <+/-15%. The deviation of the mean from the nominal value within +/-15% served as the measure of accuracy.[162] The precision and accuracy around the LoQ was acceptable if it did not exceed a CV of +/-20%.[162]

Precision, accuracy and recovery were evaluated at the experimental sample concentration. This was 5-FU (10 µg mL^{-1}), CP (400 µg mL^{-1}), DOX (10 µg mL^{-1}) and EPI (20 µg mL^{-1}). The results of precision, recovery and accuracy at the experimental sample concentration for each drug assay are shown in Table 5 on the following page. Recovery was expressed as a percentage of the standard, precision as the CV, and accuracy as the percentage range plus or minus the nominal value.

Recovery from the drug-coated surfaces was high (≥95%). The methods were reproducible and accuracy around the nominal value was within +/-15% for all drugs in all diluents.

3.5.4 Limit of Quantification and Limit of Detection

The LoQ was the lowest concentration on the calibration plot which could be quantitatively determined with reliable precision (CV = +/-20%) and accuracy (+/-20%).[162] The LoD was the smallest concentration that gave a measurable response but could not be quantified with reliable accuracy or precision. The LoD and LoQ were determined from the analysis of samples of known concentration and by visual evaluation. The LoD and LoQ for each drug assay are shown in Table 5 on the following page.

Table 5. Results of Method Validation for Recovery and HPLC Assay of 5-FU, DOX, CP and EPI, as Described in Sections 3.4.5 and 3.4.6

		Cytotoxic Drug (diluent)			
		5-FU	**CP**	**DOX**	**EPI**
Limit of Detection $\mu g\ mL^{-1}$		0.20 [a]	2.50 [a]	0.25 [a]	1.00 [a]
Limit of Quantification $\mu g\ mL^{-1}$		0.50 [a]	10.0 [a]	1.00 [a]	2.00 [a]
	Diluent				
Least-Squares Regression Coefficient (R^2)	(WFI)	0.999	0.998	1.000	0.995
	(NS)	0.999	0.999	0.999	0.990
	(pH) [b]	0.999	0.997	0.999	0.998
Inter-Day Precision CV (%)	(WFI)	3.5	3.1	0.4	8.4
	(NS)	1.9	2.2	2.5	2.6
	(pH) [b]	1.5	3.9	2.3	9.3
Intra-Day Precision CV (%)	(WFI)	1.1	5.4	1.7	6.0
	(NS)	1.3	4.5	0.4	6.4
	(pH) [b]	3.7	5.0	1.9	4.4
Mean Recovery (%)	(WFI)	98.8	96.0	99.8	96.6
	(NS)	95.9	100	99.9	96.2
	(pH) [b]	98.5	96.3	100	95.0
Range of Accuracy (%)	(WFI)	98.2 - 101	90.9 - 110	95.2 - 109	87.5 - 108
	(NS)	99.0 - 104	89.3 - 105	92.8 - 101	89.1 - 99.3
	(pH) [b]	95.7 - 104	97.6 - 110	92 .0- 98.7	97.6- 106

[a] applicable to all diluents
[b] = pH 7.0 for 5-FU, pH 3.5 for CP, pH 2.25 for DOX and pH 4.0 for EPI
CV = coefficient of variation

3.5.5 Linearity

Linearity was demonstrated over the concentration range of 0.5 to 30 $\mu g\ mL^{-1}$ for 5-FU, 10 to 700 $\mu g\ mL^{-1}$ for CP, 1.0 to 20 $\mu g\ mL^{-1}$ for DOX, and 2.0 $\mu g\ mL^{-1}$ to 150 $\mu g\ mL^{-1}$ for EPI. The average peak area of each concentration was used to construct a calibration plot for each drug. The equation for the calibration plot was

obtained by linear regression analysis of known drug concentration (*x*) versus average peak area (*y*).

The calibration plots were linear over the selected concentration range with least-squares regression analysis giving a correlation coefficient (R^2) of >0.99 (see Table 5 – previous page), indicating a good dynamic range (*x* denotes the independent variable, in this case the concentration of 5-FU, CP, DOX or EPI, and *y* denotes the dependent variable, in this case peak area). This was confirmed with visual inspection of the plot.

3.5.6 Forced Degradation Studies

5-FU, CP, DOX and EPI were degraded using forced degradation studies, to determine the parent analyte from any degradation products, in terms of retention time. 5-FU, CP, DOX and EPI (three diluents) were subjected to stress treatments under acidic, alkaline and oxidative conditions at 70^{+}/-1°C for 2 hrs. 5-FU (5.0 mg mL^{-1} × 60 µL) × 5 aliquots, CP (20 mg mL^{-1} × 250 µL) × 5 aliquots, DOX (2.0 mg mL^{-1} × 100 µL) × 5 aliquots and EPI (2.0 mg mL^{-1} × 100 µL) × 5 aliquots, were each pipetted into 10 mL volumetric flasks, and reacted separately with equivalent volumes of the following:-

a. 1 aliquot × hydrochloric acid (1.0 M) at 70^{+}/-1°C for 2 hrs,
b. 1 aliquot × sodium hydroxide (1.0 M) at 70^{+}/-1°C for 2 hrs,
c. 1 aliquot × 6 vol hydrogen peroxide at 70^{+}/-1°C for 2 hrs,
d. 1 aliquot × distilled water at 70^{+}/-1°C for 2 hrs,
e. 1 aliquot × distilled water at 4 to 8°C for 2 hrs to act as a control for the effect of heating.

All solutions were protected from light throughout. After the incubation period, each flask was allowed to equilibrate to room temperature. The acidic and alkaline solutions were neutralised by the addition of sodium hydroxide (1.0 M, 1.0 mL), and hydrochloric acid (1.0 M, 1.0 mL), respectively. Subsequently, each flask was made up to volume with Milli-Q grade water to give a final concentration of 5-FU (30 µg mL^{-1}), CP (500 µg mL^{-1}), DOX (20 µg mL^{-1}), and EPI (20 µg mL^{-1}). The final solutions were assayed by HPLC in duplicate. Peak areas were expressed as the percentage of the concentration remaining with respect to the control solution (see Table 6 below).

Table 6. Percentage of 5-FU, CP, DOX and EPI Remaining after Forced Degradation

Drug (diluent)	**Parent Drug Remaining Compared to Control (%)**			
	Acid Hydrolysis	**Alkali Hydrolysis**	**Oxidation**	**Heat (70ºC)**
5-FU (NS)	33.2	33.6	79.9	98.2
5-FU (WFI)	31.7	29.6	75.6	99.2
5-FU (pH 7.0)	31.7	22.9	79.9	96.8
CP (NS)	2.9	<LoQ	78.7	81.8
CP (WFI)	22.1	<LoQ	81.9	91.8
CP (pH 3.5)	<LoQ	<LoQ	54.8	74.1
DOX (NS)	ND	ND	75.7	90.5
DOX (WFI)	ND	ND	70.3	95.5
DOX (pH 2.25)	ND	ND	85.0	95.4
EPI (NS)	ND	ND	75.3	97.1
EPI (WFI)	ND	ND	62.9	97.3
EPI (pH 4.0)	45.4	ND	49.9	92.3

ND = none detected
LoQ = Limit of Quantification

Forced stress under acidic and basic conditions and oxidation caused degradation of the parent drug for all drug assays in all three diluents. Heating alone

had less effect. Any degradation peaks resulting from the stressed conditions were well resolved from the parent peak. DOX was sensitive particularly to acid/base-catalysed degradation, which destroyed the DOX parent peak completely in all three diluents. Under basic conditions, EPI was also destroyed completely in all three diluents. CP was degraded to below the LoQ of the analytical method by alkali hydrolysis. A degradation peak at 2.7 min was observed from CP degradation under all forced conditions and in all three diluents. This degradation peak was well resolved from the parent peak. Heating alone, had little effect on 5-FU, DOX or EPI in all diluents.

3.6 Method 2 - Development

3.6.1 Choice of Drug

CP, IFOS 5-FU, MTX, paclitaxel and platinum-containing compounds are marker drugs which have been used commonly to assess for cytotoxic surface contamination.[8] Previous studies, with the closed-system (PhaSeal®) device used CP, 5-FU and IFOS as marker drugs. These drugs are routinely used and there are analytical methods available for quantifying the surface contamination of these drugs in the environment.[8;20;62;65;67;78;124;163] For this study, three cytotoxic drugs, each from a different chemical family were selected. Two of the drugs, EPI (Figure 14, page 12) and MTX (Figure 10, page 10) were novel to investigation with the closed-system (PhaSeal®) device, and were investigated for suitability in this study. CP (Figure 2, page 4) has been investigated with the closed-system (PhaSeal®) device before,[20;62;67;78;124] thus it was investigated for its suitability for comparison with previous studies. All of the marker drugs selected for this study are in widespread clinical use in the UK.

3.6.2 Sensitivity of the Analytical Method

The aim was not only to compare the two systems for the compounding of cytotoxic drugs, but also to measure the potential risk from contamination that may be present on surfaces in an isolator and clean-room. Sensitive analytical methods were required to increase the probability of the number of samples that could be quantified above the LoD. There are no recommended safe levels of cytotoxic surface contamination; therefore, the expected levels could only be anticipated from similar studies. Contamination of EPI has not been measured extensively in the environment. One study with an LoQ of 2.0 ng mL^{-1} was sensitive to measure EPI contamination on gloves and the base of the BSC.[64] The levels of EPI contamination measured in Chapter 5 may contribute towards the paucity of EPI surface contamination data. Methods with a LoQ of 15 ng mL^{-1} [108] and 50 µg mL^{-1} [109] have been used to measure MTX surface contamination. 5-FU was the marker drug in a comparative intervention contamination study with the closed-system (PhaSeal®) device but many of the samples could not be quantified owing to the lack of sensitivity of the assay. The LoD was 20 ng mL^{-1} and the effectiveness of the device in reducing contamination could not be assessed.[124] Therefore, a sensitivity of <20 ng mL^{-1}, ideally 2.0 ng mL^{-1} or lower for EPI and MTX should be achieved.

Sensitivity of the analytical method at sub-nanogram concentrations was unnecessary to measure for CP, as nanogram levels of CP surface contamination have already been well documented and routinely measured for with the closed–system (PhaSeal®) device.[20;62;67;78;124]

3.6.3 Review of Methods for the Quantification of Epirubicin Surface Contamination

The majority of methods in the literature quantify EPI in biological matrices using reverse-phase HPLC, exploiting its fluorescence. A method using HPLC

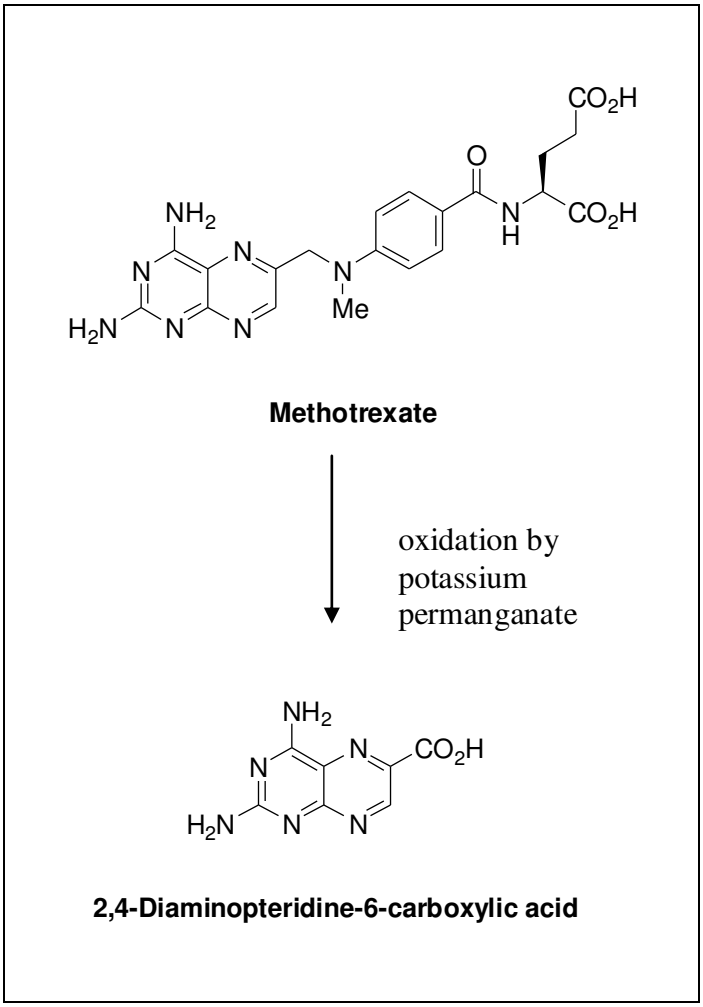
coupled with fluorescence detection at excitation and emission wavelengths of 254 nm or 480 nm, and 560 nm, respectively, with an LoQ of 0.4 ng mL^{-1} in plasma was adopted.[160] A stainless steel 250 × 4.6 mm column packed with Techsphere CN (5 µm particle size) was used. The mobile phase was phosphate buffer (0.05 M, pH 4.0) and 35% acetonitrile (v/v).[160]

3.6.4 Review of Methods for the Quantification of Methotrexate Surface Contamination

HPLC coupled with UV detection is a method used commonly for the quantification of MTX.[130] However, the LoD for one method was only 50 µg mL^{-1}.[109] MTX concentrations in biological samples are monitored very carefully in clinical practice and there are a number of different methods, which are used. These methods, like environmental levels of contamination, require high sensitivity to detect low levels of MTX. The native fluorescence of MTX is low in aqueous media but oxidation converts it to a highly fluorescent pteridine-carboxylic acid, which can be measured by HPLC with fluorimetric detection. This was the most sensitive detection method for MTX.[130]

The method adopted measures the analyte in urine by a pre-chromatographic oxidation.[164] Potassium permanganate is the oxidising agent and the reaction is quenched and the excess destroyed by hydrogen peroxide.[164-166] The documented LoQ of the highly fluorescent derivative was 10 ng mL^{-1}.[164] 2,4-diaminopteridine-6-carboxylic acid (MTX') is the oxidation product of MTX (see Figure 25 on the following page).

Figure 25. Scheme Showing the Oxidation of Methotrexate to 2,4-Diaminopteridine-6-carboxylic acid (MTX') by Potassium Permanganate



The kinetics of this reaction have been studied regarding pH, temperature and the concentration of potassium permanganate, to achieve the maximum oxidation state of MTX.[165] The method described for the oxidation of MTX to MTX' and subsequent detection was as follows:

Potassium permanganate (0.01 M, 5.0 mL) and acetic acid (0.005 M, 5.0 mL) /sodium acetate buffer (pH 5.0) were added to MTX (100 µL) in urine. The solution was vortexed for 30 sec and left to stand for 35 min at room temperature. This was the optimal time and pH for the method to achieve the highest oxidation state.[164;165]

The reaction was quenched and the excess potassium permanganate destroyed by the addition of aqueous hydrogen peroxide (33%, 0.1 mL). The pH was adjusted to 6.8 with aqueous sodium hydroxide (1.0 M) and made up to volume with mobile phase (25 mL). The mobile phase was tris hydroxymethylamineoethane (15 mM) and sodium chloride (1.0 mM) adjusted to pH 6.8 with hydrochloric acid. A stainless steel 150 × 3.9 mm column packed with C_{18} (5 µm particle size) was used, and detection was by fluorescent excitation at 280 nm and emission at 444 nm.

3.6.5 Review of Methods for the Quantification of Cyclophosphamide Surface Contamination

CP contamination has been quantified as sensitive as 0.1 ng mL^{-1} (LoD) in surface contamination studies using gas-chromatography in tandem with mass-spectroscopy mass-spectroscopy.[20;67;124] However, this method for CP is time-consuming and complicated extraction, clean-up and derivatisation is required prior to analysis. HPLC with UV detection is also used commonly. A method used in-house involving no sample pre-treatment to measure CP was adopted.[158] The method used ammonium sulfate buffer (0.005 M, pH 3.5) and 30% methanol (v/v) as the mobile phase. CP was analysed using a stainless steel 250 × 4.6 mm column packed with CN (5 µm particle size). Detection was by UV at 210 nm.[158] The LoQ for the method when used to measure CP diluted in pharmaceutical diluents was 10 µg mL^{-1}, as determined in Section 3.5.4.

3.6.6 Selection of Desorbing Solution

Optimal recovery of drug contamination requires the analyte to have a high affinity for the desorbing solution and dissolve in it. If the desorbing solution is also the storage solution the drug must be stable in it under storage conditions. The

desorbing solution for this study must also be suitable to take into a clean-room environment *i.e.* it must be sterile and non-toxic.

Desorbing solutions were considered in which all three drugs would be soluble, stable at -21°C (the temperature at which the samples would be stored after collection and prior to analysis) and be compatible with the mobile phase for the respective HPLC assay.

The analytical method for EPI was the most sensitive of the three methods, and EPI was the least stable of the three drugs. Therefore, to maintain the sensitivity of the analytical method and ensure the stability of EPI, a desorbing solution was selected which would be optimal for EPI. The methods for MTX and CP were modified to accommodate this desorbing solution. The following were considered as possible desorbing solutions:

Acetonitrile/Water (*10:90)*

Mixtures of methanol:water (50:50, 60:40 and 70:30) have been evaluated as desorbing solutions but desorption of CP from the wipe was low (~50%).[148] Mixtures of acetonitrile:water (50:50) demonstrated higher recoveries ~100% recovery for DOX (which may also be extended to EPI) but did not further improve on the recovery of CP.[148] A mixture of acetonitrile:water (10:90) recovered almost 100% of 5-FU.[150]

Normal Saline

EPI demonstrated stability for 43 days, MTX for 3 months and no significant loss of CP was observed after 4 weeks when diluted in NS and stored in infusion

devices at -20°C.[128] Longer term storage has not been investigated but repeated freeze-thawing had no significant effect on EPI stored at -20ºC.[24]

Phosphate Buffer

The stability of EPI is pH-dependent and maximum stability was observed at pH 4 to 5.[128] The HPLC mobile phase in the method adopted to assay for EPI was based on a phosphate buffer at pH 4.0.[160] MTX is almost insoluble in water but dissolves at low pH. Therefore, a desorbing solution comprising a phosphate buffer solution within the range of pH 4 to 5 would be compatible with the assay for EPI and a possible desorbing solution for both EPI and MTX.

It should be noted that phosphate has pKa values of 2.1, 7.2 and 12.3. The most effective range of a buffer is 1 pH unit either side of the pKa of the weak acid or base.[104] Therefore, phosphate is not strictly an effective buffer at pH 4.0. An acetate buffer would be a more suitable buffer at pH 4.0 as it has a pKa value of 4.8. However, it has a higher UV cut-off wavelength of 210 nm compared to phosphate buffer which has a cut-off wavelength of 200 nm.

Phosphate Buffer, Acetonitrile (30%)

EPI, MTX and CP are diverse in physical and chemical characteristics. The LogP (apparent) values in order of decreasing hydrophobicity are 3.1 (EPI), 0.2 (CP) and -0.2 (MTX).[167] Tests of various mixtures have shown that the most effective solution to desorb several drugs of different polarities from a wipe was a mixture of an organic solvent and phosphate buffer (0.01 M) pH 6.0.[148] The mobile phase for EPI comprises acetonitrile (35%) v/v. Phosphate buffer (0.01 M, pH 4.0) with acetonitrile (30%) v/v was investigated as a desorbing solution.

EPI (250 ng mL^{-1}) in each of the four desorbing solutions was assayed by HPLC and the detector response (peak area) recorded. The pH of the desorbing solution was also noted. The results are shown in Table 7 below.

Table 7. pH and Detector Response of EPI in Desorbing Solutions

Desorbing Solution	**pH**	**Detector Response (Peak Area)**
Acetonitrile:water (10:90)	6.5	2551
NS	6.5	3202
Phosphate buffer (pH 4.0)	4.0	2329
Phosphate buffer (pH 4.0), acetonitrile (30%)	4.0	4050

The largest detector response was observed when EPI was assayed diluted in phosphate buffer (0.01 M, pH 4.0) with 30% acetonitrile (v/v).

3.6.7 Stability of Epirubicin in the Desorbing Solution

EPI (250 ng mL^{-1}) was placed on storage at -21ºC for up to 12 weeks in a temperature monitored laboratory freezer. The percentage of EPI remaining after storage at 6 and 12 weeks are shown in Table 8 below.

Table 8. Amount of EPI Remaining after Storage at -21ºC in Desorbing Solutions

Desorbing Solution	**EPI Remaining after Storage at -21ºC (% of initial concentration)**	
	6 Weeks	**12 Weeks**
Acetonitrile:water 10:90	65.4	63.0
Normal saline	92.4	83.5
Phosphate buffer, pH 4.0	99.0	99.2
Phosphate buffer, pH 4.0, 30% acetonitrile	99.8	99.5

The mean value after storage was considered acceptable within +/-5% of the nominal concentration.[168] After 6 weeks of storage at -21°C, EPI was most stable in the phosphate buffer (0.01 M, pH 4.0) desorbing solutions. Phosphate buffer (0.01M, pH 4.0) with 30% acetonitrile (v/v) was selected as the desorbing solution for EPI. The conditions of the analytical method for MTX and CP were adjusted to include MTX and CP, in a single recovery method, for all three drugs using this desorbing solution.

3.6.8 Methotrexate Method Development

The method which measures MTX in urine[164] was adopted to measure MTX in desorbing solution. The reagent volumes were scaled down by a factor of 25 without changing the volume of MTX. Potassium permanganate (0.01 M, 200 µL) and acetic acid buffer (0.005 M, pH 5.0, 200 µL) were added to MTX (100 µL) in desorbing solution. After 35 mins hydrogen peroxide (30%, 60 µL) was added. The pH was adjusted to 6.2 with aqueous sodium hydroxide (1.0 M) and the sample was diluted 1:20 in mobile phase.

MTX' produces two excitation maxima located close to 280 and 380 nm, and one emission maxima at 457 nm.[165] The selected excitation wavelength was 380 nm, as the background noise recorded by the detector appeared to be visually less noisy at this wavelength. The emission wavelength was set at 458 nm (the wavelength settings on the fluorescence detector were in increments of 2).

Reverse-phase ODS silica gel is the stationary phase employed most frequently for the separation of MTX.[130] Acetonitrile is the typical organic solvent combined with an aqueous buffer ranging from pH 2.5 to 6.7.[130] The method adopted used a tris-sodium chloride buffer. However, experimentally the MTX' peak which eluted under these mobile phase conditions had a peak symmetry value of 5.49. Peak tailing was measured at 20% of the peak height, where A was the distance from peak

front to peak maximum and B was the distance from peak maximum to peak end. Ideally, chromatographic peaks are Gaussian-shaped,[161] and peak symmetry should be 1 *i.e.* A = B, but in practice, most peaks show some tailing and are acceptable within the peak symmetry range of 0.8 to 1.5.[161] The mobile phase was substituted with phosphate (0.01 M) pH 6.2 and 5% acetonitrile (v/v). This improved peak symmetry (peak symmetry value = 1.1). The scaling down of reagents and the final dilution with mobile phase had no effect on the sensitivity of the method.

3.6.9 Cyclophosphamide Method Development

For compatibility with the desorbing solution, the ammonium sulfate buffer (0.005 M) of the mobile phase was substituted with phosphate buffer (0.01 M), pH 3.1. This gave a larger detector response *i.e.* a 50 µg mL^{-1} solution of CP diluted in desorbing solution gave a detector response, in terms of peak area, of 1154 when using phosphate buffer and 316 when using the original ammonium sulfate buffer in the mobile phase.

The wavelength 210 nm is at the lower end of the UV spectrum and one where there is the possibility of other interfering compounds absorbing. To limit the absorption of other compounds which may interfere at the CP retention time window, the organic portion of the mobile phase *i.e.* methanol, was substituted with Far UV acetonitrile. This has a lower UV cut-off point at 190 nm, compared to methanol, which has a cut-off point at 210 nm. The proportion of organic solvent was modified to acetonitrile (10%). At 210 nm, absorbing peaks were observed in the recovery sample. These peaks were also observed in blank wipe recovery samples but they were not observed in the standard samples.

Resolution (R) is the measure of the degree of separation between two successively eluting components in a chromatogram. A resolution of greater than 1.5

was considered acceptable for baseline separation of the two peaks.[161] The retention time of CP was 4.7 min and the nearest adjacent peak eluted at 3.3 min. The resolution between the peak and the CP parent peak was 2.5 at 3.3 min, indicating that the peaks were resolved and did not interfere with the integration of CP.

The parameters of retention time, peak symmetry, resolution of any absorbing peaks, and sensitivity (measured as the LoQ) for EPI, MTX and CP are summarised in Table 9 below. Examples of chromatograms for each analyte are shown in Appendix 4 (Figures 55, 56 and 57).

Table 9. HPLC Assay Parameters and Sensitivity of EPI, MTX and CP

Parameters	**EPI**	**MTX**	**CP**
Retention Time	5.7 min	2.2 min	4.7 min
Peak Symmetry	1.2	1.1	1.5
Resolution from Interfering Peaks	N/A	N/A	2.5@ 3.3 min
Sensitivity (LoQ)	0.4 ng mL^{-1}	10 ng mL^{-1}	5.0 µg mL^{-1}

N/A = not applicable

3.6.10 Selection of Wipe Material and Collection Vessel

Wiping and immersion must consider the size of the container to fit the wipe or immersed surface. This will influence the volume of desorbing solution required, which will ultimately affect the sensitivity of the analytical method. The wipe or swab material must be low-linting and sterile. It must also fit into the collection vessel. Dry wipes with aqueous sodium hydroxide are used commonly for wipe sampling, whereby aqueous sodium hydroxide is pipetted onto the surface then wiped with the

dry wipe.[20;26;28;29;126] The use of a sterile impregnated wipe rather than a dry wipe was investigated. Wipes impregnated with 70% v/v alcohol (IPA or IMS) and 30% v/v WFI, are used for cleaning and controlling microorganism levels in a clean-room environment. An impregnated wipe is convenient, easier and quicker to use, ensures the equal distribution of wetting agent, leaves no residue and would reduce the amount of materials taken into the sterile environment.

In Section 4.5.2, IMS (70%) / WFI (30%) v/v was effective in removing cytotoxic contamination of 5-FU, CP, DOX and EPI from a small surface area. IPA (70%) / WFI (30%) v/v impregnated wipes have been used successfully to remove and recover surface contamination of 5-FU, a very polar molecule, with recoveries of 84.6%[149] and 90.7%.[150] Therefore, alcohol-impregnated wipes available commercially were investigated for use in this study. They varied according to surface area, weight and material - see Table 10 below.

Table 10. Specifications of the Alcohol-Impregnated Wipes Investigated

Wipe	**Wipe Composition**	**Dry Weight (gm^{-2})**	**Dimensions (mm)**
Cliniwipe	viscose rayon, styrene butadiene synthetic rubber, surfactant and antifoam 70% v/v IPA (3 mL per wipe)	22 (+/- 2.2g)	200 × 220
Spiriclens	viscose rayon, styrene butadiene synthetic rubber 70% v/v IPA	22	200 × 220
Klerwipe™	70/30 polyester cellulose blend 70% v/v IPA or IMS	22 or 42	200 × 230
Stericlean® Flowrap	polyester cellulose blend 70% v/v IPA	66	230 × 230
Stericlean® prep pad	rayon/ polypropylene mix 70% v/v IPA (+/- 0.08%)	1.1 (+/- 0.2g)	67 × 32

The material of the collection vessel (tube) must be able to withstand study storage temperatures and be compatible with the drugs and solution stored inside. EPI may bind to glass and polyethylene,[169] therefore these materials were not considered. The study for which this method was developed involved an intense sampling protocol and a high volume of wipe samples (see Sections 5.4.7 and 5.4.8), requiring 960 tubes. Considering the number of tubes required to be taken into the study area, transported and stored, they should be as small as possible. Ideally, the volume of desorbing solution should be minimal, and to prevent oxidation of the drug the headspace in the tube should also be kept to a minimum. Polypropylene centrifuge tubes (15 mL) were selected as the collection/storage vessel. Polypropylene is a suitable storage material as it can withstand temperatures of -21ºC, it is resistant to acidic pH and compatible with EPI and CP.[24]

The wipe must fit into the tube, allowing maximum contact with the desorbing solution. Wipes with a mass of 42 gm^{-2} or 66 gm^{-2} were too large and would need to be folded or rolled to fit into a tube of such a small neck diameter. This may be awkward to perform in practice and may cause the spread of cross-contamination by unavoidable contact with the wipe.

The lower weight wipes (22 gm^{-2}) *i.e.* Spiriclens, Cliniwipe, Klerwipe™ (IMS and IPA) showed significant interference with the HPLC assays. When blank samples of drug-free desorbing solution containing these wipes were injected onto the HPLC column for each assay, a peak eluted at the EPI (when excited at 254 nm) and MTX peak retention time windows. To confirm the interference, each solution was scanned using a UV spectrophotometer. A large absorbing peak from each blank wipe solution was observed in the UV region of the spectrum. The wavelength of maximum absorbance of the scan was 257 nm for the Spiriclens wipe, 268 nm for the

Cliniwipes, 271 nm for the Klercide™ wipe, and 254 nm for the Stericlean Flowrap® wipe. MTX absorbs at 280 nm,[165] EPI absorbs at 254 nm (and also at 480 nm).[160] Blank samples of desorbing solution *i.e.* containing no wipes or drug contamination, showed no interference or absorption across the UV spectrum. This confirmed that the interference observed in the chromatograms was from the wipe material. In-house studies which have used Cliniwipes to recover 5-FU, required a 1:5 dilution of the desorbate to eliminate the interference from this wipe, ultimately this affected the sensitivity of the method.[150]

Another requirement for the wipe was to have a constant size and weight to ensure consistent performance with respect to the absorption and desorption of contaminants. These data were only available for two of the six wipes *i.e.* the Cliniwipes and the Stericlean® prep pads.

The small dimensions (6.7 cm × 3.2 cm) of the Stericlean® prep pads (wipes) enabled them to be retained in a small collection tube without folding or rolling. The size of the wipe required a minimal amount of desorbing solution to cover its surface area. This would maximise recovery from the wipe and would not compromise the sensitivity of the method. This wipe did not interfere with the drug assays, compared with the other proprietary wipes. These wipes are also wrapped individually, preventing evaporation and ensuring a consistent volume of IPA.

3.6.11 Recovery of Cytotoxic Surface Contamination - Wiping and Immersion

Methods to extract the drug contamination from the wipe or the immersed surface into the desorbing solution were developed. These methods should recover the maximum amount of surface contamination with acceptable reproducibility. The recovery method was based on a procedure, used in-house, previously to measure 5-FU surface contamination in the commercial Aseptic Services Unit, Department of

Pharmacy and Pharmacology, University of Bath.[149;150] This procedure used a wipe (Cliniwipe) impregnated with alcohol (70% v/v IPA) for the removal of contamination from various surfaces. The wipe was collected into a 50 mL tube containing desorbing solution (40 mL) which was a mixture of acetonitrile:water (10:90). 5-FU contamination was removed from the wipe by vortexing the tube for 5 min, followed by centrifugation at 1500 × g for 30 min.

3.6.12 Surfaces Tested

Areas inside (including gloves) and outside of the isolator were selected from which to recover cytotoxic contamination after input from technicians, a visit to an ASU, literature review and the results presented in Table 4 (page 74).

The areas chosen inside the isolator were the base, sleeves, gloves and the hatch doors. These are areas of close proximity to the cytotoxic preparation process and are likely to be highly contaminated. The surfaces inside the isolator were made of stainless steel (base), Perspex™ (screen of the hatch door), Hypalon® (sleeves) and latex (gloves).

The areas selected in the clean-room to be measured were the floor, trays and support gloves. Contamination of CP and MTX has been measured on the floor directly in front of the isolator when preparing cytotoxic drugs,[26;28] and gloves are likely to be highly contaminated.[26;28;109] The tray is an object of high use and contact, *i.e.* for collecting and transporting consumables into the clean-room and taking items into and out of the isolator. Surfaces outside the isolator were made of vinyl (floor), plastic (trays) and nitrile (support gloves). The finished batches of syringes (polypropylene) leaving the isolator were also selected to be swabbed to identify if the contamination risk originating from the isolator may possibly be transferred to the outside environment.

A Perspex™ template with dimensions of 21 cm × 21 cm (441 cm^2) was cut and used to mark out an area on the floor, and areas of this size on the stainless surface of a BSC, the base of which is similar to that of an isolator. Portions of the surface materials were obtained; a portion of Perspex™ measuring 31 cm × 23 cm (713 cm^2) - the same area as the screen of the hatch door of the isolator, a portion of vinyl measuring 21 cm × 21 cm (441 cm^2) - the same material as the clean-room floor, nitrile gloves, latex gloves, syringes, and trays measuring 24 cm × 29 cm (696 cm^2) of the same type to be used in the study.

3.7 Method 2 - Optimisation

Phosphate buffer (0.01 M, pH 4.0) was autoclaved at 115ºC (15 psi) for 30 mins and 3.5 mL was pipetted into sterile tubes under aseptic conditions. The wipes were collected into this solution, and acetonitrile (1.5 mL) added prior to applying the recovery method.

3.7.1 Swabbing/Wiping Procedure

It was important that the designated surfaces were wiped in a methodical way to recover consistently the maximum amount of contamination. The method of wiping was developed for each individual surface and was carried out in a BSC using tongs, IPA wipes (Stericlean® prep pads), 15 mL collection tubes containing desorbing solution (3.5 mL) and the 441 cm^2 template (floor and isolator base only).

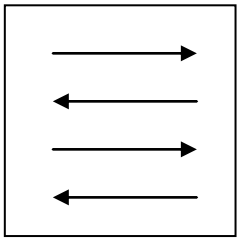
To minimise exposure to the researcher, portions of each surface were placed in the BSC and contaminated deliberately. The following solutions were pipetted onto each surface: EPI (0.1 mg mL^{-1} × 50 µL), MTX (25 mg mL^{-1} ×10 µL) and CP (4 mg mL^{-1} 1×25 µL), and allowed to dry. Using the tongs, the wipe was placed, unfolded, on the surface. Immediately, the surface was wiped by moving the wipe over it (using

tongs was safer than touching the wipe and prevented any cross-contamination from gloves).

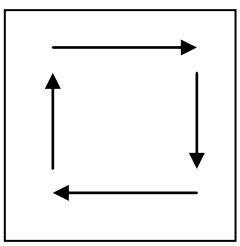
Wiping Method for the Hatch Door, Tray, Isolator Base, and Floor

The flat surfaces *i.e.* hatch door (1 wipe per side), tray (1 wipe), 3 spot areas on the isolator base (3 wipes), and the floor (1 wipe) were wiped in a four-step parallel overlapping action as shown schematically below and on the following page;

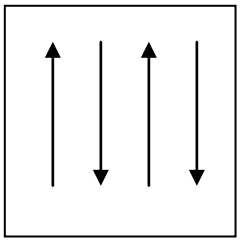
1. The area was wiped using horizontal strokes. Starting in the upper left-hand corner the wipe was moved from left to right, and back travelling downwards horizontally, covering the whole area with the wipe;



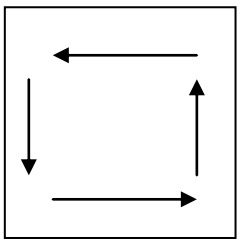
2. When arriving in the lower corner, the edge of the area was wiped around once in a clockwise direction;



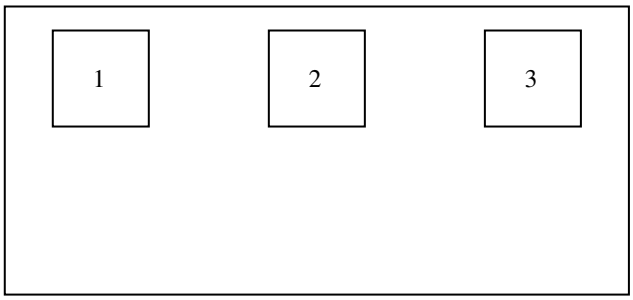
3. The same area was wiped using vertical strokes. The wipe was turned over and moved travelling across vertically and covering the whole of the area;



4. When arriving in the lower corner, the edge of the area was wiped around once again in a counter-clockwise direction.



It was not possible to swab the whole of the isolator base with a wipe of such small dimensions. Therefore, three spot areas were identified for wiping totalling 1323 cm^2 (21% of the area of the isolator base). They were 1. the far top left-hand corner; 2. the far top centre, and 3. the far top right-hand corner, as shown schematically below;



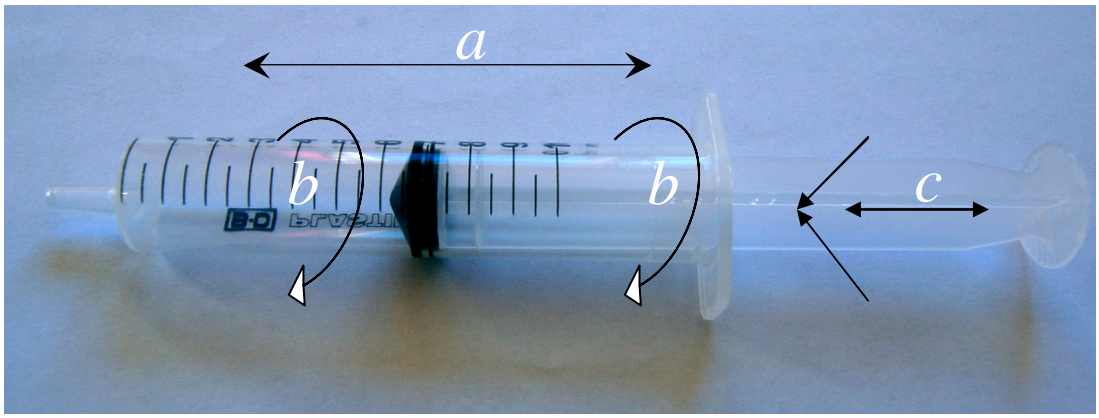
Wiping Method for the Sleeves

It was awkward to wipe the sleeve as it was not flat or rigid. Therefore, only the cuff was wiped. The left cuff was wiped with the right hand, and *vice versa*. The wipe was placed on the cuff and wiped around three times. The wipe was turned over and again the cuff was wiped around three times. One wipe was used for each cuff.

Wiping Method for the Syringes

One wipe was used each for the barrel and plunger of the syringe, and combined in the same tube. The wiping method for the syringe is shown in Figure 26 below. The wipe was moved up and down the syringe barrel three times (*a*), while turning the syringe with the other hand (*b*). The wipe was turned over and used to wipe the hub of the capped syringe. The second wipe was placed in one of the groves of the syringe plunger, in contact with both sides of the grove and moved up and down each grove three times each grove (*c*).

Figure 26. Wiping Method for the Syringe Barrel and Plunger



Each wipe was placed immediately into a collection tube containing desorbing solution (3.5 mL). The tube was sealed tightly with the cap, and the wipe was shaken

down into the solution. Acetonitrile (1.5 mL) was added and the tubes were inverted 10 times.

3.7.1.1 Recovery from the Wipe into the Desorbing Solution

Vortexing and centrifugation were investigated to extract the drug from the wipe into the desorbing solution. The additional push of the wipe and solution through a 10 mL syringe was investigated to further improve on recovery.

The following recovery methods were investigated: vortexing vigorously for 1 min; vortexing vigorously for 1 min followed by centrifugation for 5, 10, 20 or 30 min (3500 × g at 20°C) and in addition, pushing the solution and wipe through a 10 mL syringe after 30 min of centrifugation.[8;109;130]

All methods were investigated in triplicate and analysed in duplicate by HPLC. The mean value of the triplicate samples was taken and the percentage recovery calculated against a standard spiked with the same concentration. The CV was determined for each recovery method. A CV within the range of +/-15% was considered acceptable.[162] The results are presented in Table 11 on the following page.

Vortexing alone was a very poor method to extract CP from the wipe with only 4.9% recovery achieved. Centrifugation, in addition to vortexing greatly increased the recovery of CP to approximately 50%. The recovery of MTX increased with centrifugation time, with 91.8% recovered after 30 min with good precision (CV = 4.5%). In addition to vortexing, centrifugation increased the recovery of EPI, with 87.3% recovery observed after centrifugation for 30 min with excellent precision around this value (CV = 0.2%). This was a slight improvement on vortexing alone when 82.9% recovery of EPI was observed.

Table 11. Amount of Drug Recovered and Reproducibility of the Different Recovery Methods for EPI, MTX and CP

Recovery Method	**Mean Recovery of Drug (%)**		
	EPI (1 µg mL^{-1})	**MTX (50 µg mL^{-1})**	**CP (100 µg mL^{-1})**
vortex for 60 sec	82.9 CV = 3.2	63.7 CV = 14.3	4.9 CV = 7.1
vortex for 60 sec, centrifuge for 5 min	74.8 CV = 2.6	64.2 CV = 4.8	50.0 CV = 2.4
vortex for 60 sec, centrifuge for 10 min	86.8 CV = 4.8	74.3 CV = 13.8	52.3 CV = 1.0
vortex for 60 sec, centrifuge for 20 min	84.4 CV = 4.8	83.3 CV = 6.0	50.9 CV = 0.5
vortex for 60 sec, centrifuge for 30 min	87.3 CV = 0.2	91.8 CV = 4.5	50.7 CV = 2.3
vortex for 60 sec, centrifuge for 30 min, push through a 10 mL syringe	88.7 CV= 3.3	91.4 CV = 4.1	100.8 CV = 45.1

CV = coefficient of variation

The additional push of the solution though a 10 mL syringe after vortexing (1 min) and centrifugation (30 min) did not improve further the recovery of MTX, or improve significantly the recovery of EPI from the wipe. The recovery of these two drugs was already high *i.e.* EPI (87.3%) and MTX (91.8%). It did improve the recovery of CP significantly; the percentage recovered increased two-fold from 50.7% to 100.8%. However, this method could not be used to recover CP with acceptable reproducibility, as a CV of 45.1% for the method greatly exceeded the acceptable range of +/-15%. This meant that there was a large variation in the spread of the results between repeated measures. It was difficult to transfer the samples (wipe and desorbing solution) from the collection tube into the barrel of the syringe without any spillages. Loss of the desorbing solution would affect the percentage recovery calculation, giving a misleadingly higher result. Consequently, reproducibility

between samples would not be acceptable. This was also a time-consuming procedure and was not pursued further.

Centrifugation introduced a constant method with less room for operator variability between samples. With centrifugation time, an increasing amount of MTX was extracted, and a slight increase in the recovery of EPI was observed, possibly as the solution desorbed from the wipe with time by the centrifugal force applied.

Methods to increase recovery were not as successful for CP, as for EPI or MTX. A summary of analytical procedures, reported drug recoveries from environmental matrices, to range from 60 to 100%.[8] A higher recovery of CP from the wipe may have been achieved at a higher centrifuge speed however, this was the maximum safe centrifuge speed for the type of tube used.[150] A second wipe from the surface was negative for CP contamination (and for EPI and MTX), confirming that all the contamination had been removed from the surface by the wipe or at least to a level below the LoD. The percentage of CP recovered was approximately 50%, depending upon the method. This was considered low,[148] but it was reproducible.

3.7.1.2 Number of Wipes and Volume of Desorbing Solution

The feasibility of combining two or three wipes in one tube *i.e.* the three wipes taken from the isolator base, without significantly affecting the recovery from the wipes, was investigated. In combination, the probability of a sample above the LoD of the analytical method would be greater than the analysis of the samples separately. To further improve on sensitivity, smaller volumes of desorbing solution *i.e.* 2.5 mL and 2.0 mL were investigated and combined in the same experiment.

To investigate one wipe, an aliquot was pipetted equally onto one area of the base of the BSC: EPI (0.1 mg mL^{-1} × 50 µL), MTX (25 mg mL^{-1} × 10 µL) and CP (4 mg mL^{-1} × 125 µL). To investigate two wipes, two aliquots were pipetted equally onto

two separate areas of the base, and for three wipes, three aliquots were pipetted equally onto three separate areas of the base. One wipe was used for each area and all wipes were combined in a centrifuge tube containing the following volumes of the aqueous buffer part of the desorbing solution; 3.5 mL (to investigate 5.0 mL), 1.75 mL (to investigate 2.5 mL) and 1.4 mL (to investigate 2.0 mL). The following volumes of acetonitrile were added 1.5 mL, 0.75 mL and 0.6 mL, respectively. The tubes were inverted 10 times, vortexed (30 secs) and centrifuged (3500 × g at 20°C). An aliquot of the desorbing solution was transferred to autosampler vials for assay by HPLC. The tests were carried out in triplicate and analysed by HPLC in duplicate. The mean value of the triplicate samples was taken and the percentage recovery calculated against a standard spiked with the initial concentration. The reproducibility of each test was calculated and quoted as the CV. Again, a CV of +/-15% was used to show acceptable reproducibility.[162] The results are presented in Table 12 on the following page.

The mean percentage recovery of EPI, MTX and CP was highest in 5.0 mL of desorbing solution. There was no significant difference between the recovery of EPI, MTX or CP when using one, two or three wipes in this volume. Reducing the volume of desorbing solution from 5.0 mL to 2.0 mL caused a significant decrease in the amount of CP recovered from one wipe *i.e.* from 50.7% to 37.4%, respectively, and from 48.2% to 27.8%, respectively when three wipes were combined. Recoveries of CP into 2.5 mL and 2.0 mL of desorbing solution were not reproducible. The CV was greater than +/-15% when using 2 or 3 wipes to recover CP into 2.0 mL of desorbing solution, and when using 2 wipes to recover MTX into 2.5 mL of desorbing solution.

Table 12. Recovery and Reproducibility of Method Following Variation of Desorbing Solution Volume and Number of Wipes

Desorbing Volume	Number of Wipes	Mean Recovery of Drug (%) EPI (1.0 µg mL^{-1})	MTX (50 µg mL^{-1})	CP (100 µg mL^{-1})
5.0 mL	1	94.4 CV = 0.2%	91.8 CV = 4.5%	50.7 CV = 2.3%
5.0 mL	2	95.1 CV = 4.7%	98.5 CV = 3.2%	50.2 CV = 10.3%
5.0 mL	3	91.0 CV = 9.9%	98.5 CV = 2.0%	48.2 CV = 12.0%
2.5 mL	1	91.5 CV = 3.3%	86.8 CV = 9.7%	47.3 CV = 19.1%
2.5 mL	2	85.6 CV = 1.7%	79.7 CV = 17.6%	47.4 CV = 7.0%
2.5 mL	3	85.1 CV = 6.9%	69.4 CV = 12.0%	43.4 CV = 10.9%
2.0 mL	1	91.2 CV = 2.5%	84.7 CV = 1.7%	37.4 CV = 12.0%
2.0 mL	2	84.6 CV = 2.8%	75.0 CV = 2.2%	37.3 CV = 26.1%
2.0 mL	3	85.1 CV = 12.0%	82.8 CV = 11.7%	27.8 CV = 18.6%

CV = coefficient of variation

3.7.2 Immersion

Methods to measure contamination on gloves were based on immersion of the gloves in sodium hydroxide solution (100 mL)[28] or in ultra-pure water (20 mL)[68] with continual mixing for 120 min,[28] or 180 min.[68] In order not to compromise the sensitivity of the method, 10 mL of desorbing solution was investigated. The following solutions were pipetted onto each type of glove (the latex isolator glove or the nitrile support glove) in triplicate, and allowed to dry in a BSC: EPI (0.1 mg mL^{-1} × 50 µL), MTX (25 mg mL^{-1} × 10 µL) and CP (4.0 mg mL^{-1} × 125 µL). When dry,

each glove, contaminated side out was placed into a 250 mL polypropylene container. This volume of container was the smallest size available, which would fit the glove unfolded with a minimum amount of unexposed surface area. A 10 mL volume of desorbing solution *i.e.* phosphate buffer (0.01 M, 7.0 mL) pH 4.0 and acetonitrile (3.0 mL), was pipetted over the surface of the glove. The lid of the container was secured, and the container vortexed vigorously for 1 min, ensuring the desorbing solution made contact with the surface of the glove. The container was agitated continuously at 300 rpm on an orbital shaker. The variation of recovery of drug from each glove type with agitation time was investigated. An aliquot of the desorbing solution was removed and transferred to autosampler vials for analysis by HPLC. The results of recovery with agitation time are expressed in Table 13 below.

Table 13. Recovery with Agitation Time of EPI, MTX and CP from Gloves

Agitation Time (min)	**Mean Recovery of Drug (%)**		
	EPI (0.5 µg mL^{-1})	**MTX (25 µg mL^{-1})**	**CP (50 µg mL^{-1})**
Isolator glove			
30	86.9 (cv = 1.5%)	92.2 (cv = 2.8%)	50.3 (cv = 0.7%)
60	85.3 (cv = 0.9%)	93.8 (cv = 1.8%)	52.1 (cv = 1.8%)
120	88.4 (cv = 2.6%)	89.5 (cv = 3.2%)	51.9 (cv = 2.5%)
180	86.2 (cv = 3.1%)	91.7 (cv = 3.3%)	50.2 (cv = 4.4%)
Support Glove			
30	86.0 (cv = 2.1%)	89.7 (cv = 4.9%)	50.1 (cv = 4.7%)
60	84.3 (cv = 3.8%)	87.4 (cv = 1.5%)	52.5 (cv = 1.1%)
120	82.1 (cv = 3.2%)	88.6 (cv = 4.5%)	55.0 (cv = 3.6%)
180	86.9 (cv = 2.4%)	86.9 (cv = 6.2%)	51.9 (cv = 2.4%)

CV = coefficient of variation

Agitation for 30 min recovered each drug from both glove materials with acceptable reproducibility. There was no significant increase in recovery of EPI, MTX or CP

with agitation for a longer period of time from either glove material, therefore 30 min was selected as the optimal agitation time.

3.7.3 Summary of the Sampling Procedure and Final Analytical Method for EPI, MTX and CP

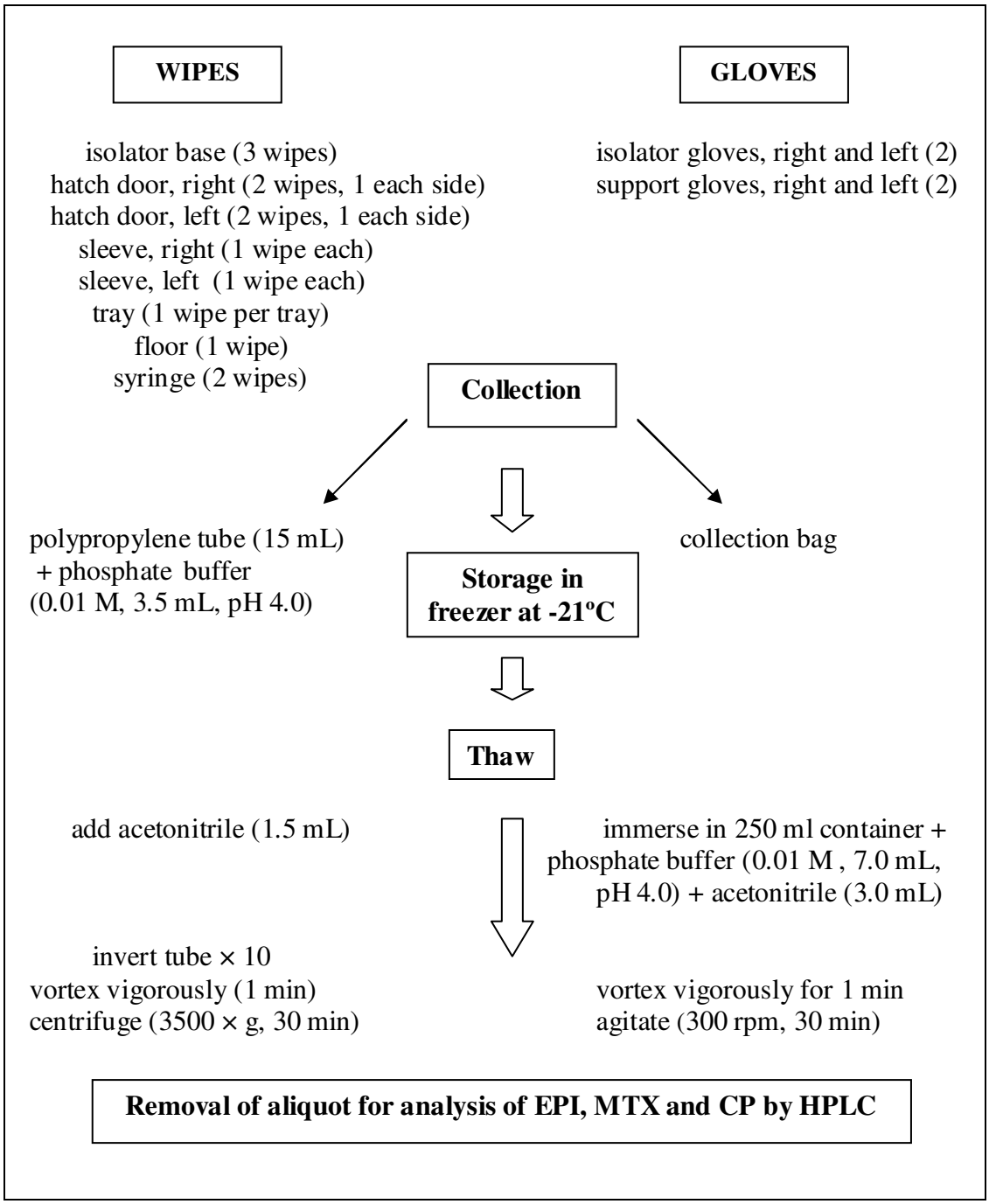
The contaminated areas (isolator base, hatch door, sleeves, trays, floor and external surfaces of syringes) were wiped, as described in Section 3.7.1. The wipes were placed into tubes containing the desorbing solution *i.e.* phosphate buffer (0.01 M, 3.5 mL) pH 4.0. Acetonitrile (1.5 mL) was added and the tubes were vortexed vigorously for 1 min, followed by centrifugation for 30 min at 3500 × g (5ºC).

Contaminated gloves (isolator and gloves) were placed into 250 mL containers. Desorbing solution (10 mL) *i.e.* phosphate buffer (0.01 M, 7.0 mL) pH 4.0 and acetonitrile (3.0 mL) was pipetted over the glove. The containers were capped and vortexed vigorously for 30 sec, followed by agitation on an orbital shaker at 300 rpm for 30 min.

The sampling procedure and recovery method used to remove EPI, MTX and CP contamination from various surfaces is summarised schematically in Figure 27 on the following page.

After centrifugation or agitation, aliquots of the desorbing solution were transferred into autosampler vials for analysis of EPI and CP by HPLC. For MTX, an aliquot of 100 µL was pipetted into a 7.0 mL tube, for the oxidation procedure of MTX to MTX'. Potassium permanganate (0.01M, 200 µL) and acetic acid buffer (0.005 M, 200 µL) were added. After 35 min, the reaction was quenched by the addition of hydrogen peroxide (30%, 60 µL), followed by aqueous sodium hydroxide (1.0 M, 38.4 µL) to adjust the pH to 6.8. The sample was further diluted 1:20 in mobile phase.

Figure 27. Schematic Summary of the Sampling Procedure and Recovery Method



The final analytical method for each drug was as follows:

EPI was analysed using a 250 × 4.6 mm stainless steel column packed with Techsphere CN (5 µm particle size). The mobile phase was phosphate buffer (0.05

M, pH 4.0) with 35% acetonitrile (v/v). Excitation was carried out at 480 nm and emission at 560 nm using a fluorescence detector. The gain was set at x1000. EPI eluted at a retention time of 5.7 min. The autosampler injection needle was flushed with 2 × 500 µL acetonitrile:water (50:50) v/v after each injection.

MTX was analysed using a 150 × 4.6 mm stainless steel column packed with Techsphere C_{18} (5 µm particle size). The mobile phase was phosphate buffer (0.01 M, pH 6.2) with 5% acetonitrile (v/v). Excitation was carried out at 380 nm, and emission at 458 nm using a fluorescence detector. The gain was set at x1000. MTX' eluted at a retention time of 2.2 min. The autosampler injection needle was flushed with 2 × 500 µL of acetonitrile:water (50:50) v/v after each injection.

CP was analysed using a 250 × 4.6 mm stainless column packed with CN (5 µm particle size). The mobile phase was phosphate buffer (0.01 M, pH 3.1) with 10% acetonitrile (v/v). Detection was by UV at 210 nm. CP eluted at a retention time of 4.7 min. The autosampler injection needle was flushed with 2 × 500 µL of acetonitrile:water (50:50) v/v after each injection.

Each column was flushed at the end of the sample run or at the end of the day. The flush solution was 10 column volumes of Milli-Q grade water, followed by either acetonitrile:water (50:50) v/v, which was also the storage solution.

3.8 Method 2 - Validation

The recovery method was validated to remove and quantify EPI, MTX and CP from eight surfaces of different material and topology *i.e.* the isolator base, hatch door, syringes, floor, tray, sleeves, support gloves and isolator gloves. Portions of each type of surface were obtained and cut or marked out to the same size of the areas

identified inside and outside the isolator that were to be investigated (see Section 3.6.12). Each area was contaminated deliberately by pipetting a volume of drug solution of known concentration over the surface and allowing it to dry in a BSC.

3.8.1 Contamination of Surfaces

Wiping was used to remove surface contamination from the isolator base, hatch door, sleeves, trays, floor and surfaces of syringes, as described in Section 3.7.1. Immersion was used to recover surface contamination from the gloves, as described in Section 3.7.2. Contamination-free surfaces were wiped or immersed and carried through the recovery process to ensure a blank response and no interference from any of the surfaces.

3.8.2 HPLC Assay

The mobile phase was prepared as described in Section 3.4.6. For each assay, elution was isocratic, the flow rate was 1 mL min^{-1}, and the injection volume was 100 µL. The recovered solutions were assayed in duplicate and the mean of the peak areas calculated. The chromatograms of all samples were processed using Prime software (Version 4.2.0).

3.8.3 Specificity/Selectivity

The specificity of each method was determined by measuring for any compounds other than the analyte of interest absorbing at the same wavelength under the chromatographic conditions for each drug. These compounds may be contaminants from the surface material or wipe, or from co-prepared drugs, which may absorb at the same wavelength. The response was measured from recovered drug-free surface samples, and from solutions spiked with the co-prepared drugs, *i.e.* the specificity of the method for EPI was tested by injecting samples containing only

MTX and CP, and *vice versa* for MTX and CP. The selectively of the method to resolve any potentially interfering compounds from the analyte peak of interest was assessed by calculating the resolution (R) between the retention times of the two adjacent peaks.

A lack of response above the LoD of the analytical method across the retention time window for EPI or MTX was observed in blank wipe samples. The presence of compounds absorbing at the same wavelength was observed on the CP chromatogram, however these were well resolved from the CP peak *i.e.* R was greater than 1.5, and did not interfere with the analysis of CP (see Section 3.6.9).

There was no interference from any co-prepared drugs *i.e.* there was no interference from the presence of MTX or CP across the EPI retention time window, and *vice versa* for MTX and CP.

3.8.4 Limit of Quantification and Limit of Detection

The LoQ of the analytical method was 0.4 ng mL^{-1} for EPI, 10 ng mL^{-1} for MTX and 5.0 µg mL^{-1} for CP. The LoD of the analytical method was 0.2 ng mL^{-1} for EPI, 5.0 ng mL^{-1} for MTX and 2.5 µg mL^{-1} for CP.

Limit of Quantification and Limit of Detection per Surface

The LoQ and the LoD per surface were expressed as mass of drug per unit of surface area *i.e.* ng cm^{-2}. The LoQ and LoD values per surface for each of the three drugs are shown in Tables 14 and 15 on the following page. These values per surface area could only be calculated for surfaces which were measurable *i.e.* the isolator base, hatch door, floor and tray. The sleeves, gloves and syringes are expressed as nanograms (EPI and MTX) or micrograms (CP) per sleeve, glove or syringe.

Table 14. Limit of Quantification of EPI, MTX and CP per Surface Sampled

Surface	Limit of Quantification per Surface		
	EPI	**MTX**	**CP**
Isolator Base	0.002 ng cm^{-2}	0.04 ng cm^{-2}	18.9 ng cm^{-2}
Hatch Door	0.003 ng cm^{-2}	0.07 ng cm^{-2}	35.1 ng cm^{-2}
Tray	0.003 ng cm^{-2}	0.07 ng cm^{-2}	35.9 ng cm^{-2}
Floor	0.005 ng cm^{-2}	0.11 ng cm^{-2}	56.7 ng cm^{-2}
Sleeve	2.0 ng per sleeve	50.0 ng per sleeve	25.0 µg per sleeve
Syringe	2.0 ng per syringe	50.0 ng per syringe	25.0 µg per syringe
Support Glove	4.0 ng per glove	100 ng per glove	50.0 µg per glove
Isolator Glove	4.0 ng per glove	100 ng per glove	50.0 µg per glove

Table 15. Limit of Detection of EPI, MTX and CP per Surface Sampled

Surface	Limit of Detection per Surface		
	EPI	**MTX**	**CP**
Isolator Base	0.001 ng cm^{-2}	0.02 ng cm^{-2}	9.50 ng cm^{-2}
Hatch Door	0.001 ng cm^{-2}	0.04 ng cm^{-2}	17.5 ng cm^{-2}
Tray	0.001 ng cm^{-2}	0.04 ng cm^{-2}	18.0 ng cm^{-2}
Floor	0.002 ng cm^{-2}	0.06 ng cm^{-2}	28.3 ng cm^{-2}
Sleeve	1.0 ng per sleeve	25.0 ng per sleeve	12.5 µg per sleeve
Syringe	1.0 ng per syringe	25.0 ng per syringe	12.5 µg per syringe
Support Glove	2.0 ng per glove	50.0 ng per glove	25.0 µg per glove
Isolator Glove	2.0 ng per glove	50.0 ng per glove	25.0 µg per glove

3.8.5 Linearity and Range

Linearity of recovery was demonstrated over the concentration range of 0.4 ng mL^{-1} to 1500 ng mL^{-1} for EPI (n= 7), 10 ng mL^{-1} to 1200 ng mL^{-1} for MTX (n= 7), and 5.0 µg mL^{-1} to 500 µg mL^{-1} for CP (n= 6). The calibration plots were linear over the selected concentration range with least-squares regression analysis giving a correlation coefficient (R^2) of >0.99 (see Table 16 on the following page), indicating a good dynamic range (*x* denotes the independent variable, in this case the

concentration of EPI, MTX or CP, and *y* denotes the dependent variable, in this case peak area). This was confirmed with visual inspection of the plot.

Table 16. Least-Squares Regression Coefficient (R^2) Values for the Recovery of EPI, MTX and CP from all Surfaces

	R^2		
Surface	**EPI**	**MTX**	**CP**
Isolator Base	0.994	0.997	0.999
Hatch Door	0.995	0.999	0.995
Tray	0.995	0.999	0.997
Floor	0.996	0.999	1.000
Sleeve	0.990	0.993	0.991
Syringe	0.996	0.997	0.994
Support Glove	0.996	0.999	0.996
Isolator Glove	0.996	0.996	0.998

The percentage recovery of each concentration from which the calibration plot was devised are shown in Table 17 (EPI), Table 18 (MTX) and Table 19 (CP) on the following pages. The least squares regression equations given were used to calculate the concentration of the drug (*x*) from the peak area (*y*), recovered from surfaces in Chapter 5.

Table 17. Recovery of EPI (%) at Concentrations Covering the Analytical Range

Nominal Concentration ($ng\ mL^{-1}$)	Surface							
	Isolator Base	Floor	Screen	Sleeve	Tray	Syringe	Isolator Glove	Support Glove
0.4	93.2	84.5	93.2	91.8	97.9	90.2	90.3	89.1
1.0	80.4	90.5	91.5	97.8	92.1	87.9	99.8	85.3
10	80.5	86.3	84.2	90.4	80.5	81.8	85.5	82.7
20	93.5	90.6	88.5	86.7	82.8	76.7	80.4	88.9
100	84.0	92.4	79.0	90.9	90.4	94.3	71.3	79.4
500	79.4	71.0	74.4	91.4	96.4	70.3	98.3	71.2
1500	78.6	72.8	98.6	73.0	90.1	97.8	91.2	77.5

Table 18. Recovery of MTX (%) at Concentrations Covering the Analytical Range

Nominal Concentration ($ng\ mL^{-1}$)	Surface							
	Isolator Base	Floor	Screen	Sleeve	Tray	Syringe	Isolator Glove	Support Glove
10	87.1	93.9	90.4	91.3	98.3	90.4	88.7	92.1
20	79.8	83.6	90.9	83.7	74.1	96.9	82.5	87.4
50	75.2	96.4	96.2	69.8	95.0	89.2	81.3	85.6
100	81.4	93.3	87.9	79.3	94.2	90.3	93.7	90.4
250	79.3	79.3	73.7	79.7	82.7	76.5	86.5	87.2
500	73.2	78.2	80.5	67.0	89.9	73.5	74.9	82.6
1000	84.1	77.9	82.6	80.7	95.4	81.8	87.7	91.3

Table 19. Recovery of CP (%) at Concentrations Covering the Analytical Range

Nominal Concentration ($\mu g\ mL^{-1}$)	Surface							
	Isolator Base	Floor	Screen	Sleeve	Tray	Syringe	Isolator Glove	Support Glove
5.0	52.6	57.1	54.9	51.7	54.9	52.6	55.1	54.7
10	50.9	54.8	56.4	53.2	56.9	52.4	53.8	54.9
20	56.9	56.0	54.2	57.8	56.2	50.9	56.3	50.7
50	58.2	56.9	53.4	52.1	56.8	50.3	54.2	59.5
100	55.8	57.2	53.0	59.4	52.1	50.9	59.9	57.5
500	54.9	56.0	55.2	54.0	51.8	53.6	52.4	55.9

3.8.6 Precision, Accuracy and Recovery

The precision, accuracy and recovery of EPI, MTX and CP at three concentrations were measured from all surfaces over the linear range of the calibration plot. This covered the highest and lowest concentration *i.e.* the LoQ of the calibration plot. EPI was measured at 0.4 ng mL^{-1}, 100 ng mL^{-1} and at 350 ng mL^{-1} or 1500 ng mL^{-1} (gloves only). MTX was measured at 10 ng mL^{-1}, 250 ng mL^{-1}, and 1200 ng mL^{-1} or 900 ng mL^{-1} (gloves only), and CP was measured at 5.0 µg mL^{-1}, 100 µg mL^{-1}, and 500 µg mL^{-1}.

The results of intra-day and inter-day precision, accuracy and recovery are shown in Table 20 (EPI), Table 21 (MTX) and Table 22 (CP) on the following pages. Recovery was expressed as a percentage of the standard, precision as the CV, and accuracy as a range of the percentage plus or minus the nominal value.

The CV of the intra-day and inter-day precision for the recovery of each drug from all surfaces were within +/-15%, and within +/-20% at the LoQ. The accuracy of the assay was within the range from 85.0 to 115%, or from 80.0 to 120% at the LoQ. The recovery from each surface was high for EPI and MTX, ranging from 70.2 to 93.5%, and 74.2 to 98.9%, respectively. The recovery of CP was lower, ranging from 50.1% to 59.8%, but reproducible.

Table 20. Recovery, Precision and Accuracy of EPI Sampled from all Surfaces

Concentration	Intra-Day (%)			Inter-Day (%)		
	Recovery	CV	Accuracy	Recovery	CV	Accuracy
Isolator Base:						
0.4 ng mL^{-1}	86.3	6.8	105.1 - 115.8	80.7	4.3	89.2 - 96.4
100 ng mL^{-1}	82.0	3.5	86.4 - 101.3	71.7	12.6	85.0 - 114.3
350 ng mL^{-1}	83.5	6.8	92.2 - 101.1	75.5	8.1	91.0 - 106.2
Hatch Door:						
0.4 ng mL^{-1}	77.7	6.0	85.6 - 99.7	80.3	11.2	89.1 - 100.0
100 ng mL^{-1}	81.8	8.7	92.6 - 106.1	76.3	13.9	91.5 - 108.8
350 ng mL^{-1}	83.0	11.4	87.7 - 95.3	71.7	14.6	87.7 - 95.6
Sleeve:						
0.4 ng mL^{-1}	77.0	7.9	101.0 - 115.6	93.5	13.5	93.6 - 110.9
100 ng mL^{-1}	84.0	6.3	92.3 - 106.5	81.7	5.2	88.7 - 94.8
350 ng mL^{-1}	78.7	10.2	95.0 - 103.1	77.7	11.4	86.8 - 111.3
Syringe:						
0.4 ng mL^{-1}	75.0	3.0	88.3 - 101.1	86.2	8.2	91.1 - 111.9
100 ng mL^{-1}	86.8	11.2	94.7 - 107.3	75.8	11.1	96.8 - 108.5
350 ng mL^{-1}	89.7	11.0	98.9 - 100.0	81.3	11.0	87.7 - 114.4
Tray:						
0.4 ng mL^{-1}	87.7	10.3	89.8 - 110.4	89.0	10.3	89.8 - 110.4
100 ng mL^{-1}	87.6	7.4	99.0 - 108.7	82.3	7.4	95.3 -105.6
350 ng mL^{-1}	70.2	5.1	97.2 - 104.8	77.1	13.5	89.0 - 107.7
Floor:						
0.4 ng mL^{-1}	82.3	6.6	103.7 - 110.4	83.8	6.6	86.4 - 93.9
100 ng mL^{-1}	84.4	3.9	93.9 - 99.4	80.2	0.8	89.9 - 90.7
350 ng mL^{-1}	85.4	3.1	91.4 - 100.0	86.5	3.1	95.6 - 100.6
Isolator Glove:						
0.4 ng mL^{-1}	85.9	7.7	92.4 -109.7	79.5	4.5	85.2 - 99.1
100 ng mL^{-1}	89.7	6.8	90.7 - 112.5	82.4	11.7	89.5 - 104.3
1500 ng mL^{-1}	81.3	4.2	95.2 - 109.8	89.7	10.2	90.3 - 106.4
Support Glove:						
0.4 ng mL^{-1}	82.1	5.3	87.5 - 103.8	80.1	8.1	91.8 - 104.7
100 ng mL^{-1}	78.4	6.2	91.5 - 108.3	86.3	5.7	93.7 - 105.6
1500 ng mL^{-1}	85.6	8.5	91.8 - 101.3	78.2	13.4	91.5 - 107.2

Table 21. Recovery, Precision and Accuracy of MTX Sampled from all Surfaces

Concentration	Intra-Day (%)			Inter-Day (%)		
	Recovery	CV	Accuracy	Recovery	CV	Accuracy
Isolator Base:						
10.0 ng mL^{-1}	92.6	9.7	86.5 - 91.5	88.6	7.3	101.5 - 115.6
250 ng mL^{-1}	98.9	10.2	88.5 - 107.0	92.0	7.0	89.1 - 105.7
1200 ng mL^{-1}	98.5	4.4	95.9 - 106.3	93.8	5.6	89.2 - 100.2
Hatch Door:						
10.0 ng mL^{-1}	86.4	6.4	94.0 - 110.0	88.3	5.3	94.0 - 113.2
250 ng mL^{-1}	82.7	2.5	99.0 - 100.2	80.2	12.5	88.5 - 107.3
1200 ng mL^{-1}	85.1	4.2	98.6 -106.2	91.3	8.0	98.9 - 104.7
Sleeve:						
10.0 ng mL^{-1}	78.3	5.3	95.0 - 106.3	84.5	5.9	83.8 - 105.0
250 ng mL^{-1}	97.8	7.0	101.5 - 115.5	90.7	0.6	100.7 - 102.2
1200 ng mL^{-1}	98.0	4.4	95.8 - 102.4	93.0	4.9	97.0 - 102.3
Syringe:						
10.0 ng mL^{-1}	74.2	12.0	89.0 - 111.0	87.5	11.6	85.5 - 101.8
250 ng mL^{-1}	89.7	4.2	88.8 - 102.9	93.6	10.4	89.5 - 102.2
1200 ng mL^{-1}	84.4	10.0	96.8 - 104.2	92.5	1.8	98.1 - 102.1
Tray:						
10.0 ng mL^{-1}	85.7	4.6	93.5 - 114.0	90.5	12.6	96.5 - 101.9
250 ng mL^{-1}	98.1	11.5	90.1 - 111.4	84.5	7.4	89.9 - 107.1
1200 ng mL^{-1}	87.5	7.2	96.1 - 106.0	82.9	4.0	94.9 - 102.5
Floor:						
10.0 ng mL^{-1}	97.0	9.2	93.3 - 114.0	93.9	6.3	96.0 - 108.0
250 ng mL^{-1}	88.1	4.4	93.5 - 107.2	91.9	8.9	92.3 - 103.4
1200 ng mL^{-1}	84.5	1.0	92.5 - 103.5	87.4	9.3	96.0 - 108.0
Isolator Glove:						
10.0 ng mL^{-1}	92.1	8.4	92.3 - 104.7	87.2	11.1	98.9 - 113.8
250 ng mL^{-1}	88.9	6.9	87.3 - 100.0	85.8	8.5	91.0 - 104.6
900 ng mL^{-1}	85.4	10.3	95.2 - 107.6	80.3	7.3	87.5 - 97.2
Support Glove:						
10.0 ng mL^{-1}	82.5	10.0	82.4 - 101.2	91.0	6.7	91.9 - 103.4
250 ng mL^{-1}	87.4	4.5	91.5 - 109.7	87.6	9.2	97.3 - 107.8
900 ng mL^{-1}	78.8	5.1	89.4 - 102.4	90.5	5.8	92.1 - 103.5

Table 22. Recovery, Precision and Accuracy of CP Sampled from all Surfaces

Concentration	Intra-Day (%)			Inter-Day (%)		
	Recovery	CV	Accuracy	Recovery	CV	Accuracy
Isolator Base:						
5.0 µg mL^{-1}	51.3	0.2	90.8 - 92.0	58.0	0.8	99.1 - 101.0
100 µg mL^{-1}	50.5	1.5	97.1 - 99.9	54.8	1.1	96.0 - 98.2
500 µg mL^{-1}	59.5	7.2	87.6 - 102.6	51.3	3.2	98.1 - 105.1
Hatch Door:						
5.0 µg mL^{-1}	52.2	1.3	98.8 - 101.0	57.2	10.4	92.6 - 100.0
100 µg mL^{-1}	59.7	4.6	92.2 - 103.2	57.9	4.6	87.5 - 97.6
500 µg mL^{-1}	59.5	4.6	8.9 - 100.7	53.3	8.4	91.6 - 111.3
Sleeve:						
5.0 µg mL^{-1}	51.5	0.5	90.2 - 91.2	52.2	7.9	90.6 - 101.1
100 µg mL^{-1}	59.0	4.2	91.4 - 100.3	58.6	1.3	89.9 - 92.6
500 µg mL^{-1}	59.5	9.1	88.3 - 110.9	52.6	9.7	91.6 - 114.2
Syringe:						
5 µg mL^{-1}	56.5	6.6	82.5 - 95.3	51.0	1.0	90.7 - 92.5
100 µg mL^{-1}	54.2	2.7	98.5 - 105.4	55.2	2.7	96.5 - 101.7
500 µg mL^{-1}	53.7	10.0	86.0 - 105.2	58.6	4.0	93.1 - 99.9
Tray:						
5.0 µg mL^{-1}	59.2	0.6	89.3 - 90.2	53.1	1.3	89.0 - 91.1
100 µg mL^{-1}	51.3	3.1	86.7 - 91.9	50.1	7.6	87.6 - 104.3
500 µg mL^{-1}	54.8	7.1	93.0 - 107.0	52.8	4.5	100.3 - 110.0
Floor:						
5.0 µg mL^{-1}	51.8	1.0	94.5 - 96.6	57.7	1.9	92.3 - 95.5
100 µg mL^{-1}	53.1	1.5	93.7 - 97.6	53.9	2.9	94.2 - 100.1
500 µg mL^{-1}	59.0	6.1	91.6 - 107.6	55.3	5.0	92.7 - 103.4
Isolator Glove:						
5.0 µg mL^{-1}	59.8	4.5	93.9 - 100.0	50.9	8.2	87.9 - 100.0
100 µg mL^{-1}	52.4	9.0	85.9 - 99.7	55.1	3.9	97.6 - 99.3
500 µg mL^{-1}	53.6	8.9	88.5 - 106.5	58.4	6.4	90.2 - 100.3
Support Glove:						
5.0 µg mL^{-1}	56.0	3.4	92.8 - 99.7	51.2	8.0	92.4 - 101.2
100 µg mL^{-1}	51.7	3.9	94.0 - 108.0	51.5	9.2	88.7 - 104.0
500 µg mL^{-1}	53.0	6.1	96.7 - 113.2	56.7	8.0	86.3 - 95.9

3.8.7 Stability

Stability should be determined over the maximum storage time, under the temperature conditions between the time of preparation and the time of sample analysis. The amount of drug degradation after storage was considered acceptable within +/-5% of the nominal concentration[168] and +/-15% if derivatisation had been carried out.[151]

The same three concentrations of EPI, MTX and CP investigated in Section 3.8.6 for precision, accuracy and recovery were investigated in triplicate. A stock solution of each concentration was diluted in phosphate buffer, pH 4.0 and transferred to 15 mL polypropylene tubes. These solutions were stored at -21ºC in a temperature monitored laboratory freezer for defined intervals over 6 months (this was the estimated time taken to analyse all of the study samples in Chapter 5). To simulate the freeze-thaw and removal of sample for the three different drug assays at each time interval, the samples were thawed, an aliquot was removed and transferred into another tube and acetonitrile (30%) was added. HPLC assay against freshly prepared standards were used to measure the percentage of drug degradation of the storage samples.

The percentage of EPI, MTX and CP, stored in phosphate buffer (0.01 M, 3.5 mL), pH 4.0 (the organic portion of the desorbing solution was not added until the experimental samples were thawed, prior to the recovery process) remaining with time at each drug concentration investigated is shown in Table 23 on the following page.

EPI was stable in the desorbing solution for up to and including 15 weeks. MTX and CP were stable in the desorbing solution for up to and including 36 weeks

when stored at -21°C and carried through the conditions of the study, from collection through to analysis.

Table 23. Storage Stability of EPI, MTX and CP in Desorbing Solution at -21ºC over 36 Weeks

Storage Interval (weeks)	Drug Remaining (%) EPI (ng mL^{-1})			MTX (ng mL^{-1})			CP (µg mL^{-1})		
	0.4	**100**	**1500**	**10**	**250**	**1250**	**5.0**	**100**	**500**
6	99.5	99.8	101.0	92.4	103.7	90.5	97.8	99.5	99.2
9	96.1	99.5	104.4	95.9	107.7	108.5	98.2	96.7	99.8
12	98.7	101.7	100.3	103.8	105.1	102.8	97.6	96.9	98.3
15	96.1	95.9	99.6	94.0	104.3	106.3	99.4	98.7	98.5
18	95.2	94.2	96.1	96.3	101.5	98.9	99.0	98.2	97.3
24	94.1	93.4	95.0	91.7	96.2	100.5	98.5	97.0	98.4
30	ND	ND	ND	97.7	92.0	97.4	97.2	97.5	98.6
36	ND	ND	ND	93.5	95.6	94.3	96.0	97.2	95.8

ND = not determined

3.9 Discussion

3.9.1 Method 1

The development of Method 1 required relatively little investigation compared to Method 2, which was more complex, requiring the drug removal and recovery of three drugs of different chemical and physical properties from several surfaces in a single method. For Method 1, HPLC methods regularly used in-house were adapted to quantify 5-FU, CP, DOX and EPI, after recovery from a test surface. The analytical methods adopted for these drugs were sufficiently sensitive to measure the levels of degradation achieved by the decontamination procedures (Chapter 4).

The aqueous part of the mobile phase specific for each drug assay was selected as the desorbing solution in which to remove and quantify each drug from the test surface (Section 3.4.4). Vortexing for 30 sec, followed by centrifugation for 5 min

at 1500 × g (5ºC) was sufficient to desorb each drug from the test surface with high recovery, and acceptable precision and accuracy (Table 5, page 100). Thus, no further investigation concerning the desorbing solution or more rigorous mechanical methods to remove the drug from the test surface were required.

The four cytotoxic marker drugs were 5-FU, CP, DOX and EPI. These served the role of marker drugs, as they are drugs of frequent use, and there were analytical methods available for quantification of all four drugs. CP was the most toxic (classified as Group 1 *i.e.* carcinogenic to humans by the IARC), followed by DOX and EPI (classified as Group 2A *i.e.* probably carcinogenic to humans).[9] 5-FU is not carcinogenic, but it is mutagenic and teratogenic, and its presence in the environment in pharmacy preparation and administration areas has been documented on numerous occasions.[20;45;61;62;66;71;125] These cytotoxic drugs were also selected because degradation by different mechanisms has been well documented for all four drugs.[5;128;153;156;170-175]

The studies that were carried out in Chapter 4, investigated the removal of cytotoxic surface contamination and degradation of cytotoxic drugs by methods, which are used currently in the pharmaceutical industry. It was important to use a chemically inert surface to carry out the tests and recover the drug from, which would not contribute towards any degradation. Polypropylene pieces cut from the barrel of 5.0 mL syringes served this purpose. The methods of degradation investigated were oxidation by VHP®, and degradation from exposure to detergents of different pH and detergents/cleaning agents of different formulations (Chapter 4).

The method was validated for all four drugs in three diluents. Two of the diluents were pharmaceutical diluents in which the drugs are likely to be diluted or reconstituted in practice *i.e.* WFI and NS, and in which form cytotoxic surface

contamination may be present. The aqueous part of the mobile phase for the corresponding drug was additionally included as a diluent. This allowed the effects of VHP® to be compared between two pharmaceutical diluents and a diluent of different pH. Forced degradation studies under stressed conditions showed that each drug assay was stability-indicating (Table 6, page 102). Peak purity measurements would have been useful to demonstrate that the HPLC peak was not attributable to more than one component *i.e.* any degradation products. Peak purity may have been determined if the spectra of the two peaks were quite different, by comparing the peak areas obtained at another wavelength, or using UV diode array detection or mass-spectrometry.[168]

The objectives of Method 1 (Section 3.2.1) were achieved and it was suitable to quantify 5-FU, CP, DOX and EPI, and measure the amount remaining on a test surface after the decontamination procedures (Chapter 4).

3.9.2 Method 2

Method 2 qualified to quantify multi-cytotoxic drug contamination from surfaces inside an isolator and from surfaces outside the isolator typically found in a clean-room (Chapter 5). The recovery method was developed for three cytotoxic drugs, each from a different chemical class *i.e.* an anthracycline antibiotic (EPI), an antimetabolite (MTX) and an alkylating agent (CP). These are drugs which pose a risk to health as they are extensively used, and are carcinogenic (CP), probably carcinogenic (EPI), or mutagenic and teratogenic (MTX).[9] The presence of MTX and CP in pharmacy preparation and administration areas has been widely reported.[20;26;58;62;63;67;68;109]

Detection methods were adopted which were sensitive to nanogram levels of EPI and MTX, and microgram levels of CP. A pre-chromatographic step involving

oxidation of MTX was necessary to achieve sensitivity as low as nanogram levels of MTX. This method required a simple derivatisation reaction and was reproducible using scaled-down volumes of reagents (Section 3.6.8).

The recovery method for Method 2 was developed to remove cytotoxic surface contamination using either wipe sampling or immersion, depending upon the type of surface. A wipe impregnated with IPA was considered for the removal of multi-surface contamination of EPI, MTX and CP. The small size of this wipe allowed a minimum amount of desorbing solution to be used *i.e.* 5.0 mL, and up to three wipes to be combined in one tube without compromising the sensitivity of the analytical method adopted. In previous in-house studies where the use of large volumes *i.e.* 40 mL of desorbing solution have been used, the sensitivity of the assay was compromised.[150]

A desorbing solution of phosphate buffer (0.01 M, pH 4.0) with acetonitrile (30%) v/v, similar to, and compatible with, the mobile phase for the EPI analytical method was selected. EPI was stable in this solution – see Table 8 (page 110), thus the analytical methods adopted for MTX and CP were modified to accommodate this desorbing solution. All three drugs were stable in the desorbing solution, had an affinity for it, and it was compatible with the HPLC assay.

The surfaces selected from which to recover cytotoxic contamination in the isolator were the main surfaces and those that were likely to be highly contaminated. Trays and gloves used outside the isolator may also be highly contaminated, as they are used to prepare for the compounding process *i.e.* gathering together the consumables and drug vials. Cytotoxic contamination has been reported on the floor when using an isolator to prepare CP and MTX.[26] Syringe batches were also chosen to be swabbed to identify if they may pick-up contamination. Contamination on the

external surface of syringe batches would transfer the risk outside of the pharmacy environment to other personnel *i.e.* those working in clinics or on hospital wards *e.g.* nurses who may handle the pharmaceutical dose during administration.

The selection of surfaces from which surface contamination was measured varied according to material, surface area and topology. The surfaces had to be consistently wiped in a methodical way to remove all of the contamination from the surface each time. Immersion was used to recover contamination from surfaces *i.e.* gloves, which would otherwise be difficult to wipe methodically. There was no significant difference between the recoveries from each surface. A lack of response across the retention time window for each parent drug peak was observed from blank surface samples (Section 3.8.3). Extra peaks from contaminants absorbing at the same wavelength were observed in the CP chromatogram. They may have come from the wipe, as they were also observed in blank wipe surface samples; however, they were well resolved and did not interfere with the CP assay.

A larger container was required for immersion of the gloves to expose as much surface area as possible to the desorbing solution to aid recovery. Contact with the desorbing solution and surface of the glove was aided by vortexing and agitation. An increase in the volume of desorbing solution from 5.0 to 10 mL observed a 2-fold decrease in the sensitivity of the assay. The immersion volume (20 mL or 100 mL) used in other studies[28;68] would have compromised the sensitivity of the assay and observed possibly a 4-fold or 25-fold decrease in sensitivity, respectively. Recovery of glove contamination into the desorbing solution was achieved after vortexing vigorously for 1 min followed by agitation at 300 rpm for 30 min. High recovery of EPI (86.9% from isolator gloves and 86.0% from support gloves) and MTX (92.2% from isolator gloves and 89.7% from support gloves), and recoveries of 50.3% from

isolator gloves and 50.1% from support gloves for CP, were reproducible for the recovery of all three drugs into 10 mL of desorbing solution - Table 13 (page 127). This gave similar percentage recoveries to the method used for recovery from the wipe samples (87.3% EPI, 91.8% MTX, and 50.7% CP) - Table 11 (page 123) therefore; a longer agitation time was not investigated.

It is more of a challenge to remove, recover and quantify the surface contamination of three structurally different drugs, than one drug, in a single method. Removal, recovery and extraction of the drug, and compatibility with the analytical method is influenced by the physical and chemical properties of the drug *i.e.* solubility, ionisation, stability. The development and validation of Method 2 achieved all the objectives set for the method – Section 3.2.2. However, the recovery method, which achieved high recoveries for EPI and MTX, recovered approximately 50% of CP. All of the CP contamination was removed by the wipe, suggesting the low recovery was due to the higher affinity of CP for the wipe and glove surface than the desorbing solution, compared to EPI and MTX. Recovery was determined by the composition of the desorbing solution, or the method used *i.e.* vortexing and centrifugation or agitation may not have provided sufficient mechanical energy to recover CP from the wipe or glove.

The impregnated wipe used in this present study was sterile, effective, and convenient to take into the clean-room environment, especially as it was already being used in the study to swab the bungs of vials. A study investigating different desorbing solutions achieved 50% recovery of CP, and 100% recovery of DOX when using a desorbing solution of acetonitrile:water (50:50). Recovery of CP was 100%, but the recovery of DOX was reduced to 60% when a desorbing solution of

acetonitrile (10%), methanol (25%) and phosphate buffer (65%) pH 6.0 v/v, was applied.[148] These desorbing solutions were pipetted onto the surface to aid recovery of the contamination. Taking a separate desorbing solution and wipe into the clean-room and isolator, and pipetting the solution onto surfaces is less convenient. It is also not practical to use this method on vertical surfaces *i.e.* the hatch door, as the solution would run off and equal distribution onto the surface would not be achieved.

MTX is a polyelectrolyte carrying two carboxyl groups with pKa values of 3.36 and 4.70. It also possesses a number of amino groups which may also dissociate; the most basic has a pKa of 5.71.[130] At pH 4.0 the carbonyl groups would be ~<50% ionised and the most basic nitrogen ~90 to 99% ionised. EPI, with a pKb of 8.2 would be ~99.99% ionised. Therefore, EPI and MTX surface contamination would be water-soluble and removed by an aqueous-based solution. In this study, a wipe impregnated with 70% IPA and 30% WFI v/v was effective in removing EPI and MTX contamination. CP is soluble in water,[24;131] and in ethanol.[131] Methanol and ethanol have a similar polarity index *i.e.* 5.1 and 5.2,[176] respectively, and are less polar than water and acetonitrile. Absorbent tissues soaked in methanol have been used to remove CP surface contamination successfully.[34] In this present study, the wipe was impregnated with IPA, which is less polar than methanol *i.e.* it has a polarity index of 3.9.[176] The addition of methanol to the desorbing solution may have increased the affinity of CP for the desorbing solution. However, altering the composition of the desorbing solution may have compromised the high recovery of EPI or MTX and may not have been as compatible with the mobile phase of the HPLC methods.

Shaking/agitation has been used to successfully recover drug contamination from gloves,[28;68] however the recovery of the method was not reported in these

studies and cannot be compared with that achieved in this present study. The highest percentage recovery of EPI and MTX was observed after 30 min of centrifugation, in addition to vortexing. MTX recovery was observed to increase significantly with centrifugation time, with 64.2% recovery observed after 5.0 min increasing to 91.8% after 30 min - see Table 11 (page 123).

Fluorimetry is more sensitive than UV detection and was employed to achieve levels of detection for EPI and MTX with an LoQ of 0.4 ng $mL^{-1}$and 10 ng mL^{-1}, respectively. EPI is a brightly coloured compound and exhibits native fluorescent properties.[160] MTX presents low fluorescence in an aqueous medium.[164] Oxidation with potassium permanganate converted this analyte into a fluorescent pteridinic carboxylic acid. The method for EPI was 5 times more sensitive than the method used in a previous surface contamination study in which the LoQ was 2.0 ng mL^{-1}.[64] The method for MTX was more sensitive (LoQ = 10 ng mL^{-1}) than other methods used for surface contamination studies *i.e.* 15 ng mL^{-1} [108] and 50 µg mL^{-1}.[109] The sensitivity which was achieved with EPI and MTX was below 20 ng mL^{-1}. In one study, an LoD of 20 ng mL^{-1} was not sensitive enough to detect 5-FU surface contamination. As a result, the surface samples could not be quantified for 5-FU, the sampling of 5-FU was terminated and the contribution of using intervention in reducing 5-FU contamination could not be assessed.[124]

The sensitivity of CP may have been improved using a method that involves a complicated multi-step sample clean-up and derivatisation, followed by gas-chromatography mass-spectroscopy. The LoD reported by this method was 0.1 ng mL^{-1}.[177] However, the intense sampling schedule of surface samples in Chapter 5 involves the measurement of 1088 samples, separately for each drug. Using this method would involve high reagent usage and specialist equipment. It would also be

considerably time-consuming, risking the analysis of all of the CP samples within the 6 months stability period at -21ºC defined in this study.

EPI may be stored at -21ºC in the aqueous buffer part of the desorbing solution for up to and including, but no longer than 15 weeks – see Table 23 (page 142). After 18 weeks, 5.8% degradation of EPI from the initial value was observed at one of the concentrations investigated. This change in drug concentration was >+/-5% and was not considered acceptable.[168] CP and MTX were more stable and may be stored for longer *i.e.* up to and including 36 weeks. During this storage time, a maximum of 4.4% and 9.5% change in assay from the initial value was observed for CP and MTX, respectively. A change in assay of +/-15% was considered acceptable for MTX to allow room for manipulation error when oxidising MTX to MTX'. On the basis of these studies the samples for EPI are analysed first within the time-frame of 15 weeks from storage, followed by the analysis of MTX' and CP within 36 weeks.

EPI and MTX were the most significant contaminants of the three cytotoxic drugs selected as marker drugs. Levels of CP contamination are plentiful in the literature – see Table 1 (page 42) and the closed-system (PhaSeal®) device has been effective in significantly reducing levels of CP contamination.[62;67;125;177] The purpose of CP was as a marker drug for comparison with other studies. The recovery of CP was lower than the other two drugs, but it was reproducible and considered acceptable for this method.

The amount of contamination produced from the compounding of cytotoxic drugs, as investigated in Chapter 5 could not be predicted, but could only be anticipated from previous surface contamination studies. Studies of this nature have reported a range from 0 to 100% in the number of positive samples.[8] Due to differences in sensitivity of the analytical methods of the three drugs, the number of

positive samples recovered from surfaces may differ. In comparison with other studies, the sensitivity of the analytical methods for the three drugs were considered acceptable and the results generated using this method satisfied the criteria of the study objectives, as described in Section 5.2. This is described in more detail in Section 5.6, where the number of positive samples and the amount of contamination of each drug recovered from surfaces in Chapter 5, using Method 2 are discussed.

3.10 Conclusion

Two methods were developed and validated to satisfy the objectives of this study. Method 1 and Method 2 are suitable to be applied to enable the quantification of cytotoxic drugs to support the analytical measurements for Chapters 4 and 5, respectively.

Method 1 was successfully developed and validated to enable the very high recovery (≥95%) and quantification of 5-FU, CP, DOX and EPI in three diluents, from a test surface with acceptable accuracy and precision. The methods were reproducible and accurate around the nominal experimental concentration (within +/- 15%), and demonstration of a linear calibration plot enabled quantification of cytotoxic drug remaining after the decontamination procedures (Chapter 4).

Method 2 achieved a sensitive LoQ at nanogram levels to remove and quantify the surface contamination of EPI (0.4 ng mL^{-1}) and MTX (10 ng mL^{-1}) and microgram levels to remove and quantify the surface contamination of CP (5.0 µg mL^{-1}). HPLC coupled with fluorimetric detection was employed as a suitable sensitive detection method for EPI and MTX. HPLC coupled with UV detection was used to quantify CP. Overall, the results show that it is feasible to remove, recover

and analyse EPI, MTX and CP surface contamination from different surfaces in an isolator and immediate environment, in single recovery method, by using wipe sampling and immersion. Surface samples contaminated with EPI, MTX and CP are stable in the desorbing solution and may be stored at -21ºC, but must be analysed within 15 weeks for EPI, and 36 weeks for MTX and CP.

Multi-surface contamination of EPI, MTX and CP can be recovered and quantified with acceptable accuracy and precision (within +/-15%, and +/-20% at the LoQ) from the isolator base, hatch door, sleeves, tray and floor using wipe sampling, and from the isolator gloves and support gloves using immersion. The recovery was high, ranging from 70.2% to 93.5% for EPI, and 74.2% to 98.9% for MTX. Recovery of CP from surfaces was lower, ranging from 50.1% to 59.8%. Linear calibration plots ($R^2 = \geq 0.99$) can be applied to quantify EPI in the range from 0.4 ng mL^{-1} to 1500 ng mL^{-1}, MTX from 10 ng mL^{-1} to 1200 ng mL^{-1}, and CP from 5.0 µg mL^{-1} to 500 µg mL^{-1} from the isolator base, hatch door, sleeves, tray, floor, syringes, isolator gloves and support gloves.

4. Decontamination of Surfaces Exposed to Cytotoxic Drugs in an Isolator Workstation

4.1 Introduction

4.1.1 Decontamination of Isolators

An isolator separates the process, which may be the compounding of cytotoxic drugs, from the operator and its surroundings. Its purpose is to provide an aseptic environment required for product protection and to protect the operator from any hazards arising from the process. Therefore, effective decontamination of the isolator is paramount to protect the product and the operator. Contamination may be in the form of biological or chemical contamination and both need to be addressed differently. Bacteria are of a known particle size,[24] while cytotoxic contaminants may be airborne or surface contamination in the form of droplets or particles. Decontamination is a combination of cleaning and disinfection, as described in Section 2.1.

4.1.2 Cleaning with Detergents

Cleaning (removal of chemical contamination) needs to be performed correctly or it may just spread contamination in the isolator. It should be methodical, *i.e.* move progressively from back to front, top to bottom, in an overlapping parallel movement. Circular movements can spread contamination and should be avoided.[110] Detergent cleaners can be either enzymatic or non-enzymatic. Non-enzymatic detergents are further classified as alkaline, acidic or neutral cleaners and are recommended for cleaning an isolator.[110] The importance of detergents is that, unlike single chemical solutions, they are composed of a series of components such as acid/alkali/neutral base, pH regulators, oxidising agents, chelators, surfactants and

solubilising agents. These components work together to clean a specific target soil from a surface. WFI is used typically to remove detergent residues/surfaces films after detergent application and is the water quality recommended for isolators.[110] The detergents used in this study were formulated to meet the cleaning demands found in the hospital and pharmaceutical industry.[178]

4.1.3 Disinfection by Vaporised Hydrogen Peroxide

Disinfection (removal or elimination of microbiological contamination) can be achieved using direct or indirect methods. Direct methods apply a liquid disinfectant *e.g.* biocides, phenolics, alcohols onto a surface by spraying or wiping. Indirect methods deliver antimicrobial gas or liquid fumigants indirectly to the surfaces of an enclosed area.[110]

VHP® is a fumigation method which is more effective at lower concentrations than liquid hydrogen peroxide.[110;147] It is applied widely in the hospital environment where it is used as a surface decontaminant of enclosed areas and for medical device sterilization.[147] VHP®, a strong oxidising agent, is an odourless, colourless gas produced from the vaporisation of liquid hydrogen peroxide. The advantages of VHP® over more traditional fumigants such as formaldehyde are that it is safe to use and breaks down to non-toxic products *i.e.* water and oxygen.[110] There are two hydrogen peroxide vapour processes 'wet' and 'dry' based on the physical chemistry of the hydrogen peroxide applied. The wet process introduces gaseous hydrogen peroxide at high concentrations, which on reaching its dew point condenses onto surfaces. Condensation of peroxide results in varied non-standard delivery, which is difficult to validate. The wet process requires a long cycle time, specifically the aeration phase required to remove the condensed peroxide. The dry process introduces gaseous hydrogen peroxide and maintains it below its dew point *i.e.* non-

condensed. The dry process results in a vapour, which is maintained throughout the decontamination phase producing a standard form of VHP® delivery to the surface. In addition to this, cycle time is reduced, achieving effective disinfection in less time than that required for traditional fumigation methods. The 'dry' process has demonstrated superior antimicrobial activity compared to the 'wet' process and is the method of choice for the decontamination of hard surfaces such as in isolators and clean-rooms.[110] VHP® has a broad spectrum of antimicrobial properties. It has been demonstrated to be bactericidal, sporicidal, fungicidal, and efficacious against organic molecules *i.e.* endotoxins and proteinaceous-based toxins.[147;179-181] However, its effect on cytotoxic drugs is unknown and there are no reported studies. This study investigates the effect of VHP® dry process on cytotoxic drugs.

4.1.4 The VHP® 100P Bio-Decontamination Unit

The STERIS VHP® bio-decontamination system generates and delivers hydrogen peroxide vapour to sealed enclosures, providing a consistent fumigation process. Cycle times vary with initial temperature and humidity, and are calculated for a given area to ensure condensation does not occur. The cycle operates in a closed-loop configuration consisting of four phases, which are monitored continuously by internal sensors and controlled with pre-programmed process parameters:

i. the dehumidification phase reduces the humidity within the enclosure to a pre-determined level to prevent condensation;
ii. the conditioning phase involves injecting VHP® into the enclosure so that the required VHP® concentration is achieved;

iii. the decontamination phase maintains the desired VHP® concentration at a constant level, by continually introducing the vapour into the incoming air and degrading VHP® catalytically to water vapour and oxygen in the returning air;

v. the aeration phase evacuates VHP® rapidly. It is complete when the concentration of VHP® is at, or below, 1 ppm.

The decontamination of four marker drugs was investigated in this study. Three drugs were used initially; they were 5–FU, CP, and DOX. In addition, studies were extended to include EPI. The rationale for inclusion of these drugs are described in Section 3.4.1.

4.2 Objectives

The objectives of this study were to investigate the safe decontamination of cytotoxic drugs from surfaces of a pharmaceutical isolator workstation. The effects of two decontamination technologies available in the pharmaceutical and healthcare environments *i.e.* liquid detergents/cleaning agents and VHP® were evaluated on cytotoxic marker drugs in two phases:

- Phase I investigated the physical removal of the drugs by cleaning with aqueous-based detergents of different pH and cleaning agents;

- Phase II investigated:

  i. the breakdown of the drugs by the same detergents and cleaning agents,

  ii. the ability of VHP® to degrade the drugs by oxidation.

4.3 Materials

Cytotoxic Drugs

5-Fluorouracil 25 mg mL^{-1} (lot N012669) was obtained from Mayne Pharma Plc, Leamington Spa, UK. Cyclophosphamide, Doxorubicin and Epirubicin were obtained, as described in Section 3.3.

Chemicals

Liquid hydrogen peroxide (Vaprox®) (lots 0-2021092185-BF, 02004104135BF and 02021046135BF) was supplied by STERIS, Basingstoke, UK

Detergents/Cleaning Agents

The detergent concentrates CIP 100 (lot 231630), CIP 150 (lot 233946), Criti-Klenz (lot 2318326L3), Renu-Klenz (lot 234091364), NpH-Klenz (lot 236024), Cage-Klenz 250 (lot 223611), CIP 200 (lot 232809) and CIP 220 (lot 229960) were supplied by STERIS, Basingstoke, UK.

Klercide 70/30 sterile denatured ethanol (lot 051005EP), Klerclean sterile neutral detergent (lot 060203ND/4) and Klercide CR sterile filtered biocide B (lot 060310BP/4) were obtained from Shield Medicare, Farnham, UK.

Equipment

The VHP® 100P generator (serial no. 0135103–29), VHP® 100P High Output generator (serial no. 0118705-05), flexible-walled isolator (serial no. 013510-29), and exposure chamber were supplied by STERIS, Basingstoke, UK.

Consumables

Chemdi chemical indicators (lot 227519/1/A), hydrogen peroxide detection tube and the Accuro® pump (serial no. 8101041) were supplied by STERIS, Basingstoke, UK. The Stericlean® low-linting dry wipes (lot W016603) were obtained from Helapet Ltd, Bedfordshire, UK.

4.4 Methods

Cytotoxic drug preparations, surface coating of test surfaces and Phase I and IIi of the study were carried out in a BSC. Exposure of the drugs to VHP® (Phase IIii), Cycle 1 was carried out in a flexible-walled isolator, Cycles 2 and 3 were carried out in an exposure chamber. HPLC analysis and all tests were carried out at the University of Bath, UK, except for VHP® exposure Cycles 2 and 3, which were carried out at the University of Cardiff, Wales.

4.4.1 Drug Dilution/Reconstitution

All four-marker drugs were reconstituted or diluted in three diluents; WFI and NS, and the aqueous component of the mobile phase specific to the drug assay (see Section 3.4.2). The third diluent was included to evaluate the study of the decontamination procedure at different pH. Glucose was not used as it has been suggested that it may consume some of the oxidising potential of hydrogen peroxide.[113]

Phase I, which involved removal by wiping, was carried out on the drugs diluted or reconstituted in WFI and NS. Phase IIi, which involved degradation by detergents, was carried out on the drugs diluted or reconstituted in WFI. Phase IIii, which involved degradation by VHP®, was carried out on the drugs diluted or reconstituted in all three diluents.

4.4.2 Test Surface Coating

The test surfaces were coated (Phase I and IIii), as described in Section 3.4.3, by pipetting 10 µL or 20 µL of drug solution onto the concave side of the surface. To eliminate the effect of the drying process and the temperature reached inside the BSC on drug stability, drug controls were allowed to dry alongside the test samples. Surface controls of coating with drug-free diluent only and blank non-coated surfaces were also included. All test surfaces (including the controls and blanks) were allowed to dry in the BSC for 2 hrs (until no solvent remained). For Phase I, the surfaces were treated in the BSC. For Phase IIii, the surfaces were placed horizontally in the centre of the isolator or exposure chamber on a flat plastic tray elevated at approximately 30 cm or 10 cm above base level, respectively.

4.4.3 Immersion and Recovery of Drug from the Test Surface

Immersion methods were used to remove and recover as much drug as possible from the test surface for Phase I and IIii tests. The recovery method was as described in Section 3.4.5.

4.4.4 LC Methods for Drug Quantification

Validated HPLC methods, as described in Section 3.4.6 were used to quantify the amount of the parent drug 5-FU, CP, DOX or EPI after all study phases. Quantification was conducted using the external standard method; duplicate test injections were bracketed by injections of standard solutions. The concentrations of the standard solutions were 5-FU (10 µg mL^{-1}), CP (400 µg mL^{-1}), DOX (10 µg mL^{-1}) and EPI (20 µg mL^{-1}). These were the same concentrations as the initial concentration of the experimental samples applied to the test surfaces.

4.4.5 Chemical Composition of the Detergents (formulations)

Eight detergents (acid, alkali and neutral) covering an extreme pH-range (referred to as the pH-detergents), and two cleaning agents used commonly in hospital pharmacies for the decontamination of isolators, were investigated for the purpose of this study. The major components of the formulated detergent/cleaning agents are shown in Table 24 below.[182]

Table 24. Chemical Composition of the Detergents and Cleaning Agents Included in this Study

Detergent/Cleaning Agent	pH	Chemical Components of Formulation
pH-detergents		
CIP 220	acidic	<30% w/v hydroxyacetic acid
CIP 200	acidic	<50% w/v phosphoric acid <30% w/v citric acid
Cage-Klenz	acidic	<30% w/v citric acid
Renu-Klenz	neutral	high levels of surfactants
NpH-Klenz	neutral	high levels of surfactants
Criti-Klenz	alkaline	high levels of surfactants <20% w/v EDTA
CIP 150	alkaline	<10% w/v potassium hydroxide <1% w/v sodium hydroxide <5% w/v sodium hypochlorite
CIP 100	alkaline	<25% w/v potassium hydroxide <5% EDTA
Cleaning Agents		
Klerclean neutral detergent	neutral	a non-ionic fatty alcohol ethoxylate
Klercide CR-B	alkaline	blend of stabilized chlorine dioxide and QACs

The acidic detergents were formulated with acids i.e. citric, hydroxyacetic, phosphoric. The alkaline detergents were formulated with sodium hypochlorite and claimed to have the oxidising properties of chlorine, or were formulated with sodium hydroxide and/or potassium hydroxide. Some detergents were formulated with surfactants, chelating or sequestering agents to aid the removal of surface soiling. They were non-corrosive and formulated at concentrations which were suitable for cleaning the surface materials of an isolator.[182]

4.4.6 Phase I - Physical Removal by Detergents and Cleaning Agents

The ability of the detergents and cleaning agents to remove dried cytotoxic drug contamination from a coated test surface were investigated. The eight detergents of different pH were used in a wipe study to remove 5-FU, CP and DOX and the two cleaning agents were applied to remove 5-FU, CP, DOX and EPI. WFI and IMS were used as the controls. For each detergent/cleaning agent, 50 µL was pipetted onto a cytotoxic-coated surface and control test surface. This was the optimal volume to cover the contamination for the size of area to be wiped, simulating cleaning in practice. Wiping involved one stroke across the test surface. The area was wiped immediately using a dry wipe cut to the dimensions of 2.0 cm × 6.0 cm. If drug remained on the surface *i.e.* the amount remaining was above the LoD of the method, a second surface treated with the same drug was wiped twice. If still above the LoD of the analytical method, a third surface of the same drug was wiped three times. Any remaining drug was recovered from the wiped test surface and analysed by HPLC.

4.4.7 Phase IIi - Deactivation by Detergents/Cleaning Agents

The ability of the detergents/cleaning agents to degrade cytotoxic drugs was investigated using a modification of the suspension test as set out in British Standard (BS EN 1656; 2000).[183] A liquid hydrogen peroxide (35% v/v) solution (Vaprox®) was also included to compare with VHP®. A solution (100 µL) of test drug was mixed with an equal volume (100 µL) of detergent/cleaning agent or Vaprox® at room temperature. The test drugs for reaction were 5-FU (5 mg mL^{-1} × 100 µL), CP (4 mg mL^{-1} × 100 µL) - diluted in WFI from the 20 mg mL^{-1} working concentration, and DOX and EPI (1 mg mL^{-1} × 100 µL). The solution was vortexed for 1 min then incubated for up to 60 min at 22 to 23°C. After incubation, the solutions were diluted with the aqueous component of the mobile phase (800 µL). 5-FU, DOX and EPI were further diluted in the respective mobile phase to achieve the experimental sample concentration prior to immediate assay. IMS was used as a control.

4.4.7.1 pH Measurement of Detergents and Cleaning Agents

The pH of the detergents and cleaning agents were measured in-house using a glass electrode and pH meter (see Table 25 on the following page). The eight detergents of different pH subjected the drugs to extreme levels of pH ranging from 1.7 to 13.2. The detergents were liquids and were diluted in distilled water according to manufacturers' instructions. The cleaning agents were in the form of liquid sprays, which were used as supplied.

Table 25. Dilution and pH of the Detergents, Cleaning Agents, Vaprox® and IMS

Detergent	Dilution	pH
pH-Detergents		
CIP 100	0.8	13.2
CIP 150	0.8	12.8
Criti-Klenz	1.6	11.3
Renu-Klenz	1.6	8.0
NpH-Klenz	1.6	7.5
CIP 220	1.6	2.4
Cage-Klenz	1.6	2.3
CIP 200	1.6	1.7
Cleaning Agents		
Klerclean neutral	N/A (as supplied)	4.7
Klercide CR-B	N/A (as supplied)	9.5
Vaprox®	35% v/v (as supplied)	2.6
IMS	70% v/v (as supplied)	3.5

N/A = not applicable

4.4.8 Phase IIii - Deactivation by VHP®

VHP® delivery and control systems have been developed to provide a consistent fumigation process for a given enclosure. VHP® was generated using Vaprox® inside a VHP® 100P bio-decontamination mobile unit, which when connected to the isolator or exposure chamber controlled the whole dry fumigation process. The conditions for the four phases of each cycle are given in Table 26 on the following page.

Table 26. VHP® Cycle Conditions for Cycles 1, 2 and 3

Parameter	Value		
	Cycle 1	**Cycle 2**	**Cycle 3**
Dehumidification			
airflow ($m^3 h^{-1}$)	18	28	28
absolute humidity (mg L^{-1})	2.3	2.3	2.3
time (min)	10	10	10
Conditioning			
airflow ($m^3 h^{-1}$)	12	18	18
injection rate (g min^{-1})	2.5	1.0	1.6
time (min)	3.0	1.0	1.0
Decontamination			
airflow ($m^3 h^{-1}$)	12	18	30
injection rate (g min^{-1})	1.6	1.2	1.6
time (min)	25	120	120
Aeration			
airflow ($m^3 h^{-1}$)	18	28	28
time (min)	90	120	120

Cycle 1 was carried out in a flexible-walled isolator with an area of 1 m^3. 5-FU, CP and DOX were exposed to 1.6 g min^{-1} of VHP® for 25 mins during this cycle. The parameters of Cycle 1 were modified to give a longer exposure time during Cycles 2 and 3. This was possible using an exposure chamber and a High Output Unit, which were able to operate to maintain the concentration of VHP® during a longer period of time.

Exposure time was increased from 25 min to 2 hrs, and VHP® was applied at a concentration of 1.2 g min^{-1} (Cycle 2). Cycle 2 was applied only to CP to determine the extent of CP susceptibility to oxidation with time.

The concentration of the 2 hr cycle (Cycle 2) was increased from 1.2 to 1.6 g min^{-1} (Cycle 3) and applied to 5-FU, CP, DOX and EPI. Cycle 3 determined the extent of susceptibility of 5-FU to oxidation with a longer exposure time, and CP

with increased VHP® concentration in comparison with Cycle 2. DOX was exposed to investigate further degradation with a longer exposure time. In addition, EPI was exposed to extend oxidative susceptibility to a drug from the same chemical family as DOX.

Prior to each cycle, a leak test was carried out to ensure that the flexible-walled isolator or exposure chamber were leak-proof. During the leak test and three exposure test cycles, regular VHP® gas measurements were carried out using a hand held hydrogen peroxide detection tube in the vicinity of the equipment, to ensure the absence of VHP® leakage. Chemical indicators were placed evenly within the inside of the isolator or exposure chamber to confirm the semi-quantitative presence of VHP® throughout the enclosure. The indicators were printed with blue-grey indicator ink, which is responsive to VHP®, and a strip of control ink identical in colour to the unexposed ink. Once exposed to VHP® the indicator ink turned to a beige colour within 15 mins, for comparison with the control ink which did not change colour.[184] A printer readout from the VHP® generator recorded the cycle parameters and injection concentration of VHP® in the enclosure, at 1 min intervals, during the course of each cycle. In addition, room temperature and the temperature inside the enclosures were recorded and signs of condensation monitored. If condensation had appeared, the cycle would have been aborted. The study was set up and carried out in a standard dry laboratory with good natural lighting. To eliminate the effect of light on drug stability, control samples were placed outside the isolator/exposure chamber during the cycles. To eliminate the effect of the temperatures reached inside of the isolator or exposure chamber on drug stability, control samples were exposed to a blank cycle minus VHP®. After exposure to VHP®, the drugs were recovered from the test surfaces (Section 3.4.5) and quantified by HPLC (Section 3.4.6 and 4.4.4).

4.5 Results

4.5.1 Test Surface Coating

The drying process, applied after coating the test surfaces with drug in the BSC, had no effect on the drug controls. In addition, no effect was observed on surface controls coated with drug-free diluent only, or on blank non-coated surfaces.

4.5.2 Phase I - Physical Removal by Detergents and Cleaning Agents

Table 27 below, shows the number of wipes required to remove the drug contamination from the surfaces by the various detergents and cleaning agents.

Table 27. Physical Removal of 5-FU, CP, DOX and EPI by the pH-Detergents and Cleaning Agents

	Number of Wipes Required to Remove Drug							
Test	**5-FU (WFI)**	**5-FU (NS)**	**CP (WFI)**	**CP (NS)**	**DOX (WFI)**	**DOX (NS)**	**EPI (WFI)**	**EPI (NS)**
WFI	1	3	1	1	1	1	1	1
IMS	1	1	1	1	1	1	1	1
pH-Detergents								
CIP 100	1	1	1	1	2	2	-	-
CIP 150	1	1	1	1	3	3	-	-
Criti-Klenz	1	1	1	1	2	2	-	-
Renu-Klenz	1	1	1	1	1	1	-	-
NpH-Klenz	1	1	1	1	1	1	-	-
Cage-Klenz	1	1	1	1	1	1	-	-
CIP 220	1	1	1	1	1	1	-	-
CIP 200	1	1	1	1	1	1	-	-
Cleaning Agents								
Klercide CR-B	1	1	1	1	1	1	1	1
Klerclean neutral	1	1	1	1	2	1	1	1

5-FU and CP were easily removed from the test surface when using a dry wipe, with acid, neutral or alkaline detergent. DOX was removed easily by acid or neutral detergent, but was more persistent to removal by alkaline detergents, requiring more than one wipe to remove all traces (not quantifiable but above the LoD of the analytical method). An immediate colour change from red to purple was evident when DOX was exposed to the three alkaline detergents. The depth of purple increased with increasing alkalinity of the detergents. This colour change was not observed with the controls (WFI and IMS).

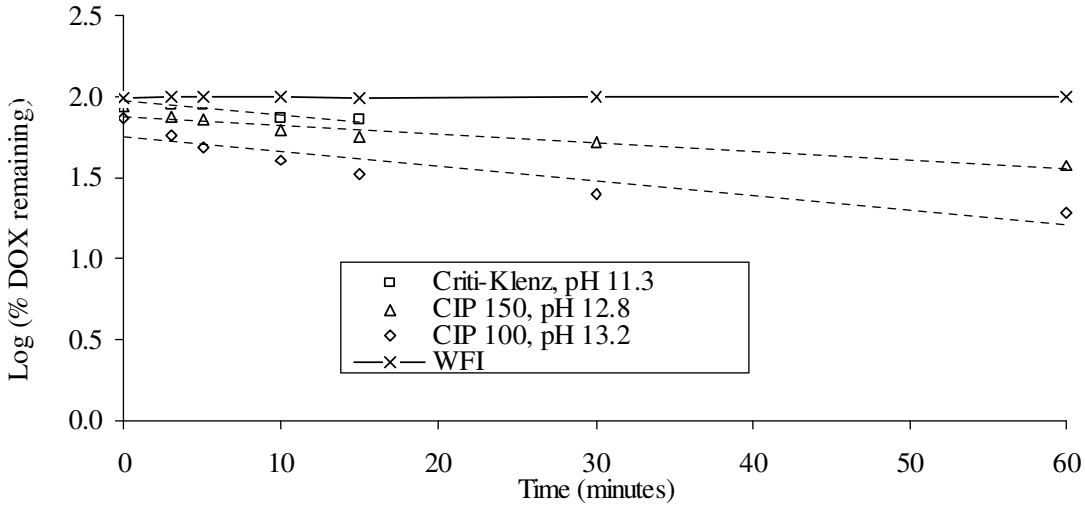
Wiping with WFI and IMS was effective in removing 5-FU, CP and DOX. All detergents were superior to WFI when removing 5-FU diluted in NS.

The cleaning agents easily removed 5-FU, CP, DOX and EPI. Only one wipe across the surface was required, except for DOX (WFI), which was slightly more persistent and required two wipes with Klerclean neutral detergent.

4.5.3 Phase IIi - Deactivation by Detergents and Cleaning Agents

5-FU and CP were not susceptible to chemical decomposition across the pH range of 1.7 - 13.2, after 60 min of exposure to all detergents and liquid hydrogen peroxide. DOX was not susceptible to degradation at acid and neutral pH for up to and including 60 min. However, significant degradation of DOX was observed with alkaline detergents. Exposure for 15 min to CIP 100 caused 66.5% degradation, CIP 150 caused 44.2% degradation and Criti-Klenz caused 28.5 % degradation. Exposure of DOX for 60 min caused 81.0% degradation by CIP 100 (pH 13.2) and 62.3% by CIP 150 (pH 12.8). The rate increased with increasing alkalinity of the detergent formulation. The rate of degradation of DOX by alkaline detergents is shown in Figure 28 on the following page.

Figure 28. Semi-Log Plot Showing the Amount of DOX Remaining with Time after Exposure to the Alkaline Detergents CIP 100, CIP 150 and Criti-Klenz



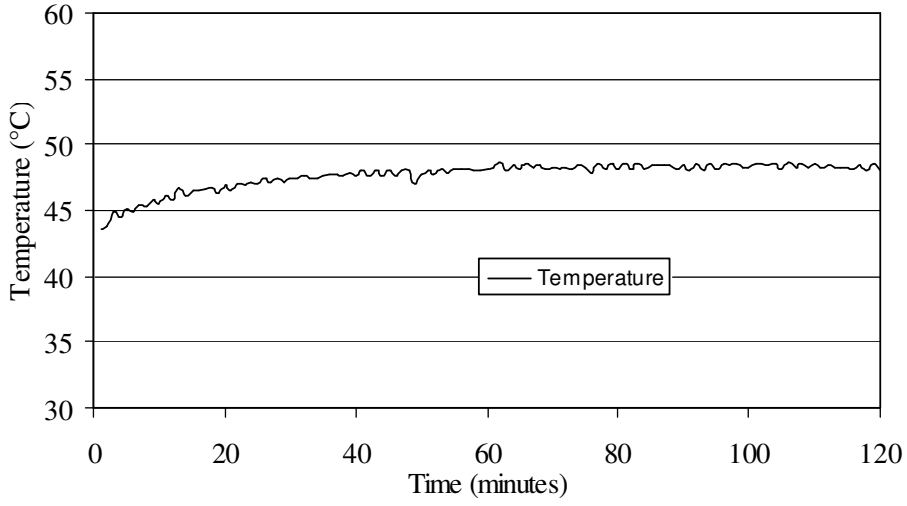
The rate of degradation of DOX when exposed to Criti-Klenz can be described by the equation $y = -0.0089x + 1.9773$ ($R^2 = 0.934$) with a half-life of 31.2 min. The rate of degradation of DOX by CIP 150 can be described by the equation $y = -0.0055x + 1.8847$ ($R^2 = 0.904$) with a half-life of 33.6 min. The rate of degradation of DOX by CIP 100 can be described by the equation $y = -0.009x + 1.7485$ ($R^2 = 0.847$) with a half-life of 5.4 min. All three drugs demonstrated no degradation after exposure to liquid hydrogen peroxide for 60 min.

5-FU, CP, DOX and EPI were not susceptible to degradation by the cleaning agents. Klerclean neutral detergent, although branded a 'neutral' detergent, measured a weakly acidic pH. Temperatures, which did not exceed 24ºC throughout the study, had no effect on the controls and therefore did not contribute towards the degradation of DOX observed.

4.5.4 Phase IIii - Deactivation by VHP®

All three decontamination cycles with the VHP® were completed successfully. The chemical indicators changed colour from blue-grey to beige within 15 mins confirming the semi-quantitative presence of VHP®. This was evident throughout the flexible isolator and the exposure chamber, and indicated a successful validated cycle. During Cycle 1, internal isolator temperature readings averaged 30ºC, and external air temperature did not rise above 28ºC. During Cycle 2, the internal temperature of the exposure chamber averaged 42.3ºC, and the external air temperature readings did not exceed 25ºC. During Cycle 3, the internal temperature inside the exposure chamber averaged 46.7ºC (range 43.6 to 48.7°C). The temperature recorded throughout Cycle 3 is shown graphically in Figure 29 below. External air temperature readings did not exceed 26°C.

Figure 29. Graph Showing Internal Temperature (°C) Recorded in the Exposure Chamber during Cycle 3



No condensation occurred inside the isolator or the exposure chamber at any point throughout the duration of the three exposure cycles. VHP® had no effect on the blank surfaces or drug-free diluent controls in the study. Table 28 below shows the amount of 5-FU, CP and DOX remaining after exposure to Cycle 1, expressed as a percentage of the initial concentration, plus or minus the standard deviation. The results are from the exposure of triplicate samples and subsequent analysis by HPLC in duplicate.

Table 28. Assay Results for 5-FU, CP and DOX after Exposure to VHP® at 1.6 g min^{-1} for 25 Minutes (Cycle 1)

Drug (diluent)	**Exposure**	**Drug Remaining (%) after Exposure +/-SD**
5-FU (WFI)	VHP®	97.9 +/- 0.5
5-FU (NS)	VHP®	96.5 +/- 1.2
5-FU (pH 7.0)	VHP®	99.1 +/- 0.2
CP (WFI)	VHP®	98.7 +/- 9.2
CP (NS)	VHP®	97.7 +/- 15.2
CP (pH 3.5)	VHP®	94.1 +/- 10.4
DOX (WFI)	VHP®	56.6 +/- 0.4
	control (light)	102.4 +/- 0.1
	control (temperature)	101.0 +/- 0.5
DOX (NS)	VHP®	44.0 +/- 0.4
	control (light)	98.9 +/- 0.6
	control (temperature)	100.0 +/- 0.6
DOX (pH 2.25)	VHP®	8.1 +/- 0.4
	control (light)	90.1 +/- 0.3
	control (temperature)	97.9 +/- 0.5

SD = standard deviation

Under the conditions of Cycle 1, VHP® had little or no effect on 5-FU. The concentration of 5-FU remaining after VHP® exposure was 97.9% (WFI) and 96.5% (NS) of the initial concentration. This was slightly lower than the range of accuracy of the method *i.e.* the range of accuracy for 5-FU (WFI) was from 98.2 to 101%, and 99.0 to 104% for 5-FU (NS) - see Table 5 (page 100). This was not considered significant since no degradation products or significant decreases in analyte peak height were observed.

Similarly, VHP® had no significant effect on CP. The amount of CP (pH 3.5) remaining after VHP® exposure was 94.1% which was slightly lower than the range of accuracy of the method *i.e.* the range of accuracy for CP (pH 3.5) was from 97.6 to 110% - see Table 5 (page 100). Again, this was not considered significant as no degradation products or significant decreases in peak height were observed.

VHP® did cause significant degradation of DOX. The amount of degradation was dependent on the diluent used *i.e.* 43.4% degradation with WFI (pH 5.9), 56.0% with NS (pH 6.5) and 91.9% with sodium chloride (0.01 M, pH 2.25). No colour change of DOX was observed with VHP® exposure. Furthermore, there was no evidence of degradation of DOX in the controls from the effect of temperature, or any significant effects from natural light throughout the cycle duration. The concentration of the light control of DOX (pH 2.25) after exposure was 1.9% lower than the accuracy of the method – see Table 5 (page 100). This may be due to limited degradation of DOX from light exposure throughout the duration of the cycle. However, in comparison to the amount of degradation caused by VHP® (91.9%), this was not considered to be significant. A significant reduction was observed in the parent DOX peak, which eluted at 3.8 min. There were also additional peaks eluting at 2.3 min and at 4.7 min. These eluting peaks were degradation products of DOX.

They were not identified or quantified. See Appendix 4, Figures 58 and 59, for chromatograms showing of the degradation of DOX (buffer) following exposure to Cycle 1.

Under the conditions of Cycle 2, CP was not susceptible to oxidation after 2 hrs exposure, at a concentration of 1.2 g min^{-1}. Table 29 below shows the amount of CP remaining, expressed as a percentage of the initial concentration, plus or minus the standard deviation after exposure to Cycle 2. The results are from the exposure of triplicate samples and subsequent analysis by HPLC in duplicate.

Table 29. Assay Results for CP after Exposure to VHP® 1.2 g min^{-1} for 2 Hours (Cycle 2)

Drug (diluent)	**Exposure**	**CP Remaining (%) after Exposure +/-SD**
CP (WFI)	VHP®	104.4 +/- 4.2
CP (NS)	VHP®	104.7 +/- 1.2
CP (pH 3.5)	VHP®	105.7 +/- 6.1

SD = standard deviation

The conditions of Cycle 3, caused the degradation of three out of the four drugs when exposed in all diluents, and of 5-FU when exposed diluted in NS or buffer (pH 7.0). Table 30 on the following page shows the amount of 5-FU, CP, DOX and EPI remaining after exposure to Cycle 3, expressed as a percentage of the initial concentration plus or minus the standard deviation. The results are from the exposure of triplicate samples and subsequent analysis by HPLC in duplicate.

Table 30. Assay Results for 5-FU, CP, DOX and EPI after Exposure to VHP® at 1.6 g min^{-1} for 2 Hours (Cycle 3)

Drug (diluent)	Exposure	Drug Remaining (%) after Exposure +/-SD
5-FU (WFI)	VHP®	98.5 +/- 3.8
	control (light)	102 +/- 7.5
	control (temperature)	99.8 +/- 0.3
5-FU (NS)	VHP®	70.7 +/- 5.9
	control (light)	95.1 +/- 2.2
	control (temperature)	103.7 +/- 3.2
5-FU (pH 7.0)	VHP®	84.9 +/- 0.2
	control (light)	98.1 +/- 6.6
	control (temperature)	95.7 +/- 6.7
CP (WFI)	VHP®	1.1 +/- 0.17
	control (light)	87.5 +/- 1.2
	control (temperature)	97.0 +/- 6.1
CP (NS)	VHP®	6.8 +/- 1.1
	control (light)	89.4 +/- 0.3
	control (temperature)	90.5 +/- 0.4
CP (pH 3.5)	VHP®	25.4 +/- 0.2
	control (light)	98.2 +/- 0.1
	control (temperature)	100.2 +/- 6.4
DOX (WFI)	VHP®	30.4 +/- 1.4
	control (light)	96.8 +/- 3.1
	control (temperature)	96.2 +/- 1.2
DOX (NS)	VHP®	29.0 +/- 6.0
	control (light)	92.3 +/- 4.9
	control (temperature)	93.3 +/- 4.7
EPI (WFI)	VHP®	34.1 +/- 0.4
	control (light)	106.5 +/- 0.8
	control (temperature)	104.8 +/- 3.3
EPI (NS)	VHP®	69.0 +/- 2.1
	control (light)	98.3 +/- 1.6
	control (temperature)	98.8 +/- 3.2
EPI (pH 4.0)	VHP®	45.5 +/- 4.5
	control (light)	103 +/- 4.2
	control (temperature)	99.2 +/- 5.3

SD = standard deviation

VHP® had little or no effect on 5-FU (WFI) under the conditions of Cycle 3. Exposure of 5-FU (NS) caused 29.3% degradation and 15.1% when diluted in buffer (pH 7.0).

The longer exposure time of Cycle 3, caused significant degradation of CP. CP (WFI) was slightly more susceptible to degradation, with only 1.1% of drug remaining *i.e.* VHP® caused 98.9% degradation, followed by CP diluted in NS (6.8% remaining) and CP diluted in pH 3.5 buffer (25.4% remaining).

An increase in the degradation of DOX (WFI) from 43.4% (Cycle 1) to 69.6% (Cycle 3) was as a result of the longer exposure time. Furthermore, 56.0% degradation of DOX (NS) during Cycle 1 increased to 71.0% during Cycle 3. The effect of Cycle 3 on DOX (pH 2.25) was not determined as DOX demonstrated limited stability at pH 2.25. DOX was stable *i.e.* within +/- 5%[168] for up to and including 5 hrs of storage in sodium chloride (0.01 M, pH 2.25); at 6 hrs 6.1% degradation was observed. Due to the logistics of the study i.e. the time taken for transport after a 4 hr cycle, the results in Table 30 (page 173) show exposure of DOX in the pharmaceutical diluents only.

EPI was also degraded by VHP®. During Cycle 3, 65.9% degradation of EPI (WFI) was observed, a similar effect was observed on DOX in WFI (69.6%). EPI (NS) compared to DOX (NS) was less susceptible to oxidation with only 31.0% degradation occurring compared to 71.0%, respectively. When EPI was exposed diluted in buffer (pH 4.0) 54.5% degradation was observed.

There was no evidence of degradation of CP, or EPI in the control test surfaces from the effect of temperature or natural light throughout the cycle duration. The concentration of the light control for 5-FU (NS) was slightly lower (95.1%) than the range of accuracy of the method (99.0 to 104% - see Table 5, page 100). VHP®

caused 29.3% degradation of 5-FU (NS) in the test samples therefore, the contribution of natural light towards degradation was minimal. VHP®, light or temperature had no effect on blank surfaces or on any of the diluents used as blank controls.

No significant degradation products, observed as additional peaks, were present in the chromatograms showing the degradation of 5-FU or CP. A small peak at 3.6 min present in a chromatogram representing the degradation of EPI may be a degradation product of EPI. It was not identified or investigated further. There was no evidence of degradation of EPI diluted in WFI, NS or pH 4.0 buffer in the controls from the effect of natural light or temperature throughout the cycle duration.

4.6 Discussion

This study was set up initially as a pilot study, to investigate the effect of current decontamination technologies (liquid detergents/cleaning agents and VHP® fumigation) as potential agents, which could reduce the risk to the operator by reducing or eliminating drug contamination from a surface.

Three cytotoxic marker drugs (5-FU, CP and DOX) were exposed to detergents of different pH and cleaning agents (Phase I and IIi), and VHP® (Phase IIii). Based on the results, these tests were further extended to EPI, another drug likely to be pH sensitive and susceptible to degradation by oxidation.

Preliminary wipe tests with strong alkaline, acid, or neutral detergents, and wiping with a dry wipe removed all drugs tested from a contaminated surface – see Table 27 (page 166). WFI and IMS, the controls, were also as efficient. 5-FU (NS) was more persistent to removal by WFI than 5-FU (WFI), requiring 3 wipes compared to 1 wipe, respectively. 5-FU is formulated with sodium hydroxide to prevent precipitation which may occur at pH <8.[128] Effectively, the 5-FU is present as

a sodium salt. The presence of sodium ions in the formulation and in the diluent (NS), could have reduced the solubility of 5-FU sodium salt in solution, as a result of the common-ion effect. The reduced solubility would make 5-FU (NS) more persistent to removal than 5-FU (WFI).

The solubility of a drug depends upon the extent to which it is ionised. This is determined by the pKa of any acidic and basic groups and the pH of the environment. Drugs are more soluble and likely to be taken up by a wipe at the pH at which they are 100% ionised. DOX has a pKa value of 8.2[171] and would have been ionised and very water-soluble at the pH it was subjected to by the acidic detergents (pH 1.7 to 2.4) and not at the pH of the alkaline detergents (pH 11.3 - 13.2). This could explain why it was easier to remove from the surface with acid rather than alkali detergents; although, alkaline detergents played a part in the degradation of DOX. Persistency to removal at alkaline pH, indicates that surfaces contaminated with DOX would need to be cleaned twice with detergents/cleaning agents of alkaline pH, or extra pressure applied to remove all of the contamination from the surface.

Wipe tests with the two cleaning agents used currently to decontaminate isolators and a dry wipe were also effective in removing all drugs, including EPI. DOX was slightly more persistent to one of the cleaning agents.

Exposure of drug solution for up to 1 hour in strong acid and alkali and neutral-based detergents caused no degradation of 5-FU or CP. Degradation of DOX occurred when it was subjected to detergents of alkaline pH after 1 hour of exposure. The exposure of DOX to alkaline-based detergents resulted in an immediate colour change of DOX from red to purple, which is indicative of alkaline decomposition.[24]

CIP 100 (pH 13.2) caused the most degradation of DOX, 81% after 1 hour, with 50% occurring after 5.4 min - see Figure 28 (page 168). CIP 100 is formulated with potassium hydroxide and is 0.4 of a pH unit more alkaline than CIP 150, which is formulated with potassium hydroxide, sodium hydroxide, and sodium hypochlorite (<5% w/v). DOX has been reported to be degraded completely by sodium hypochlorite (5.25% w/v) after 1 hour of exposure.[115;185] The strong alkalinity of the detergent appeared to be the dominant factor in determining degradation of DOX rather than oxidation *i.e.* by sodium hypochlorite, indicating that hydrolysis was pH-dependent. DOX was also susceptible to VHP®, indicating that the degradation of DOX occurred through two different mechanisms, oxidation being one method and alkali hydrolysis being the other. No colour change was observed with oxidation.

Exposure for up to 1 hour to the cleaning agents which are used for the decontamination of isolators after the compounding of cytotoxic drugs caused no degradation of 5-FU, CP, DOX or EPI. Klercide CR-B would be used in cleaning for its biocidal properties but it would not be effective in degrading cytotoxic contamination of any of the four drugs tested in this study.

5-FU and CP were not susceptible to the initial VHP® parameters applied in Cycle 1 – Table 26 (page 164). DOX was subject to oxidation by VHP®. The degree of degradation was dependent on the diluent used, with significant degradation observed when DOX was exposed following dilution in a strongly acidic diluent (pH 2.25) – see Table 28 (page 170). Further studies carried out to investigate the effects of VHP® over a longer exposure time subjected the drugs to harsher conditions. To maintain the concentration of VHP® for Cycles 2 and 3, it was necessary to use an exposure chamber, as flexible-walled isolators leak with time.[186] As a result, the

drugs were also subjected to higher temperatures. VHP® penetration of the target increases with temperature and it would be expected to be significantly more effective at higher temperatures. Drug controls exposed in the isolator/exposure chamber to a blank cycle subjected the drugs to the same temperatures minus VHP® exposure. These controls confirmed stability to the temperatures reached during the cycles. Initial exposure to VHP® carried out at 1.6 g min^{-1} for 25 min (Cycle 1) had no significant effect on 5-FU or CP, which was expected, as these are drugs not likely to undergo oxidation. Increasing the length of exposure from 25 min to 2 hrs at a lower concentration of 1.2 g min^{-1} (Cycle 2) was not effective against CP – see Table 29 (page 172). The increase in VHP® from 1.2 to 1.6 g min^{-1} (Cycle 3) under the same conditions, *i.e.* time and temperature, had a significant effect – see Table 30 (page 173). CP was susceptible to degradation by this small increase in concentration. The longer exposure time and increase in concentration of Cycle 3 compared to Cycle 1 increased the amount of degradation of DOX. It may appear from Table 30 (page 173) that VHP® Cycle 3 had a greater effect on CP compared to the other drugs. However, quantification of the drugs cannot be compared between methods due to the difference in sensitivity of the HPLC assays. The method for DOX was a 10-fold more sensitive assay compared to the method for CP – see Table 5 (page 100). Although the CP peak was almost degraded completely the measurement was only slightly above the LoD of the CP assay, which was 2.5 µg $mL^{-}1$, compared to 0.25 µg mL^{-1} for DOX. Cycle 3 also caused degradation of EPI. 5-FU was the least susceptible to oxidation of the four drugs. However, degradation (29.3%) of 5-FU was observed when it was exposed diluted in NS. When exposed diluted in WFI the drug solution was not susceptible to oxidation. A significant difference in the amount of degradation was also observed between the pharmaceutical diluents when DOX

was exposed diluted in NS, compared to WFI (Cycle 1). An increase in ionic strength by the addition of sodium chloride to a solution has been demonstrated to increase the rate of degradation of DOX.[171] The rate of degradation of DOX by VHP® in this study may have been increased by the influence of the ionic strength of NS as the diluent. This may have also influenced the rate of degradation of 5-FU, and would explain why more degradation of DOX and 5-FU was observed when exposed diluted in NS compared to WFI.

DOX and EPI were the least stable to oxidation. Cycle 3 further increased the degradation of DOX in the pharmaceutical diluents by 26.2% when exposed diluted in WFI and by 15% when exposed diluted in NS. However, DOX was not degraded completely to below the LoD for the analytical method. The susceptibility of DOX to oxidation is reported in the literature,[116;153] DOX and EPI are also unstable at pH values less than 3 or greater than 7.[128] In this study, DOX was susceptible to oxidation by VHP®, and its instability at a pH lower than pH 3 was confirmed.

This study confirms the superior stability of 5-FU and CP compared to DOX, since no decomposition occurred during exposure to liquid hydrogen peroxide or even when exposed to the extreme pH conditions (pH 1.7 to 13.2) applied. 5-FU and CP were not susceptible to oxidative stress during initial exposures to VHP® but CP was more susceptible to the harsher exposure conditions.

EPI was less susceptible to oxidation than DOX. Cycle 3 caused more than twice as much degradation of DOX (71.0% degradation) compared to EPI (31.0% degradation) when exposed diluted in NS. When exposed diluted in WFI the amount of degradation between the two drugs was similar. Between the three diluents, EPI was most stable in WFI (pH 5.9) and not at pH 4.0. The amount of degradation

between the drugs when exposed at different pHs values cannot be compared. DOX was exposed in a pH at which it was unstable after a certain period of time, and EPI, CP and 5-FU would demonstrate some stability at the respective pH of the aqueous part of the mobile phase. Comparisons could have been made if the drugs had all been exposed in standard buffers covering a range of pH values. However, surface contamination of these drugs in an isolator would only be found in the pharmaceutical diluents and further work should investigate these more relevant diluents only.

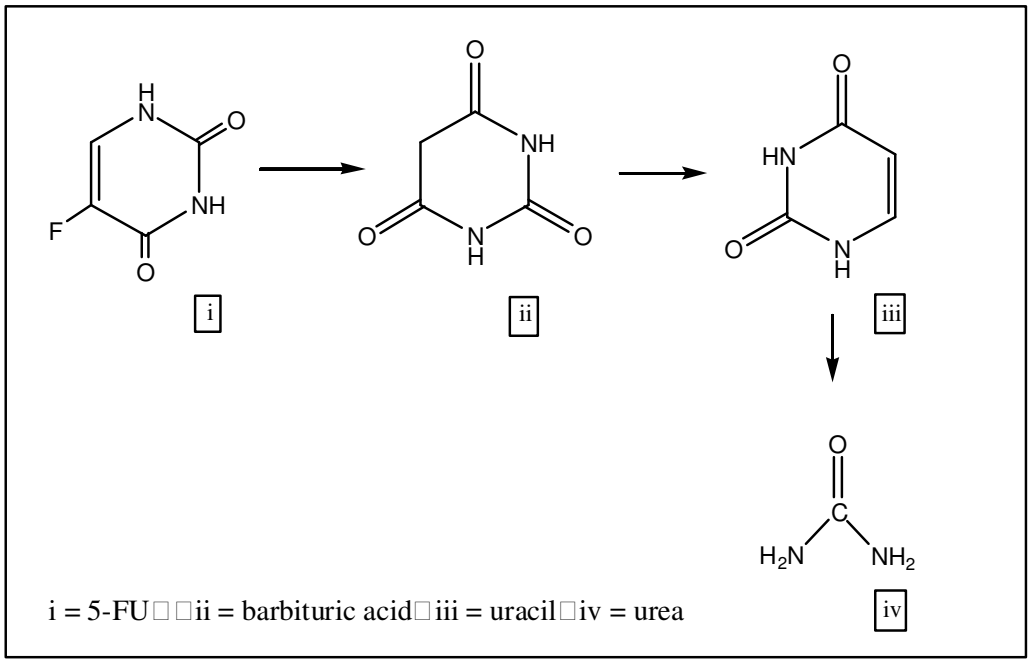
The gaseous form of hydrogen peroxide is advantageous over the liquid form as it contacts all the surfaces that it is exposed to, even those of complex topographies, ensuring a uniform decontamination. The gaseous form of hydrogen peroxide was demonstrated to be a more effective oxidising agent than liquid hydrogen peroxide, which in this present study had no effect on the decomposition of DOX. The ineffectiveness of liquid hydrogen peroxide in degrading DOX has already been reported.[115]

Liquid hydrogen peroxide (35%) and CIP 150 formulated with sodium hypochlorite (<5%) had no effect on CP. These results are not in total agreement with the literature.[113;115] Sodium hypochlorite (5.25%) and ≤30% liquid hydrogen peroxide have been reported to exhibit >98% efficiency in inactivating CP to non-mutagenic residues after 1 hour of exposure,[113] with complete disappearance of the parent peak when assayed by HPLC. However, the method used was not as sensitive; any contribution to cytotoxicity could only be measured from residues equivalent to 20 µg of degraded CP tested per plate in the Ames mutagenicity tests, and the LoD of the analytical method was 20 µg mL^{-1}, compared to an LoD of 2.5µg mL^{-1} in this present study.

Exposure to potassium hydroxide (0.2 M) in methanol solution for one hour is recommended for the chemical destruction of CP.[24] The detergent formulations CIP 150 and CIP 100 contain <10 % w/v (<0.02 M) and <25% w/v (<0.04 M) potassium hydroxide, respectively. Detergents formulated with a higher concentration of potassium hydroxide may have been more effective in degrading CP.

Phase I was carried out following a review of drug structures. Alkaline hydrolysis above pH 9.0 appears to be the likely method of degradation of 5-FU in solution.[5] The range of alkaline-based detergents had no effect on 5-FU after 1 hour. Certainly, the alkaline hydrolysis of 5-FU is a slow process.[5] The degradation of 5-FU by alkaline hydrolysis is shown schematically in Figure 30 below.[5]

Figure 30. Scheme Showing the Hydrolysis of 5-Fluorouracil at Alkaline pH[5]

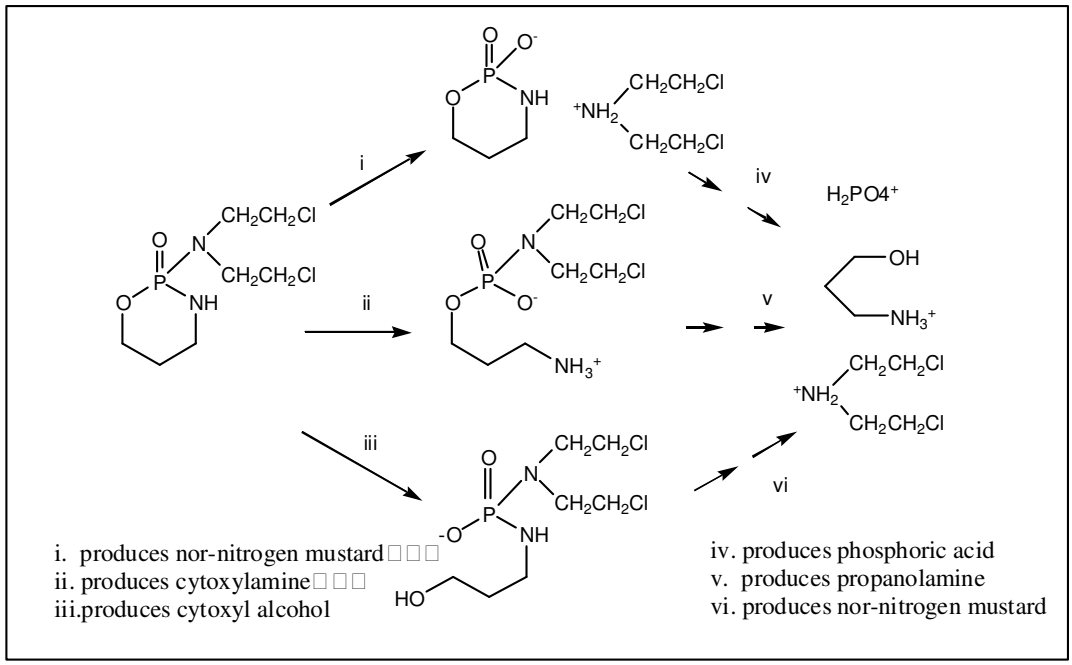


Degradation of 5-FU (i) produces barbituric acid (ii), which degrades more rapidly than it is formed to uracil (iii), which then degrades to the end product urea

(iv).[5] Thermal and photo degradation also lead to this end product,[24] which is not cytotoxic. In this study, the thermal and photo stability of 5-FU was confirmed with the temperature and light controls.

Although, CP mainly degrades by hydrolysis *in vitro*,[172;173;187;188] chemical degradation by oxidation has also been reported. Oxidation of CP by Fenton's reagent produced acrolein,[175] which is one of the cytotoxic metabolites produced from the enzymatic oxidation of CP in the liver.[1] The rate of CP hydrolysis in aqueous solution is constant over the pH range of 2 to 10. Specific acid and specific base catalysis occurs at extreme pH.[5] Initial acidic hydrolysis may occur in one of three ways to produce intermediate products which are alkylating agents: i. cleavage of the exocyclic N-P bond to release nor-nitrogen mustard; ii. cleavage of the endocyclic N-P bond to produce cytoxylamine or iii. cleavage of the endocyclic O-P bond to produce cytoxyl alcohol.[5;173] This is shown schematically in Figure 31 below.[5]

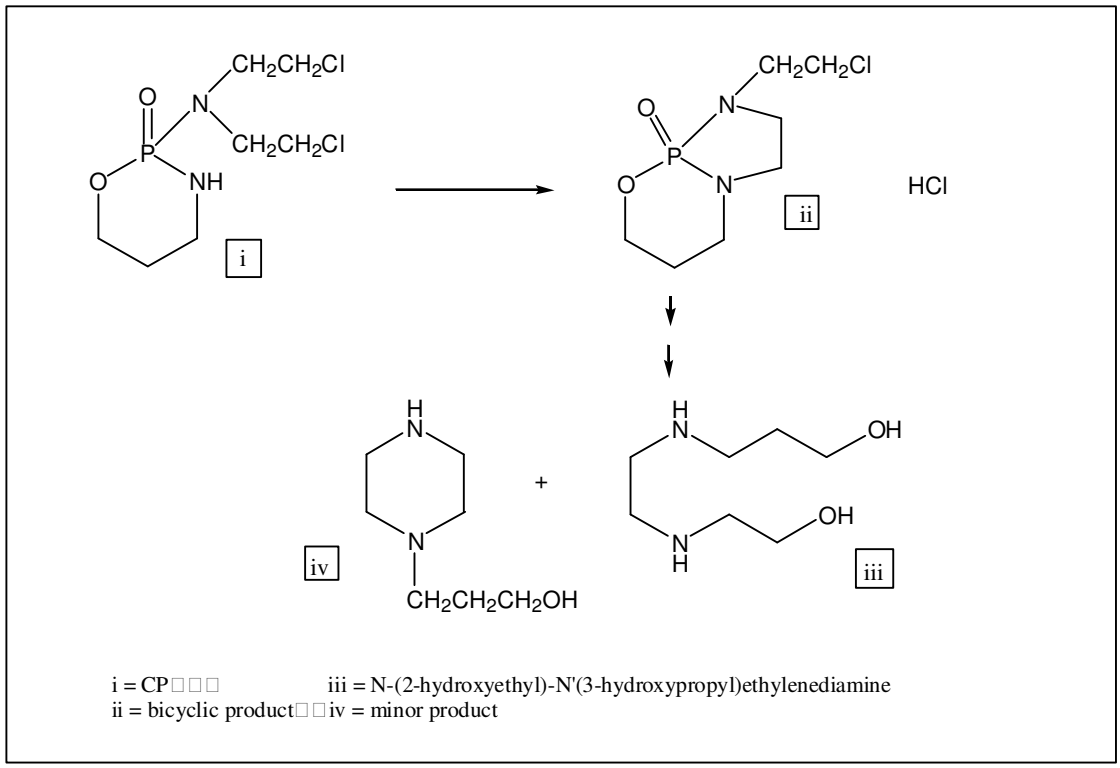
Figure 31. Scheme Showing the Three Possible Hydrolysis Pathways of Cyclophosphamide at Acidic pH[5]



Subsequent hydrolysis of these intermediate products produces phosphoric acid (iv), propanolamine (v) and nor-nitrogen mustard (vi).

The scheme for the degradation of CP at neutral or alkaline pH is shown in Figure 32 below.[5] Under neutral or basic conditions, CP (i) hydrolysis occurs by an initial intra-molecular N-alkylation with the loss of a chloride ion that forms hydrochloric acid, and a bicyclic product (ii).[187] This compound is very labile in aqueous solution and undergoes a series of hydrolytic cleavages of amide (N-P) and ester (P-O) bonds to produce the main product iii. N-(2-hydroxyethyl)-N'(3-hydroxypropyl)ethylenediamine and iv. a minor product from a second alkylation.[173;187]

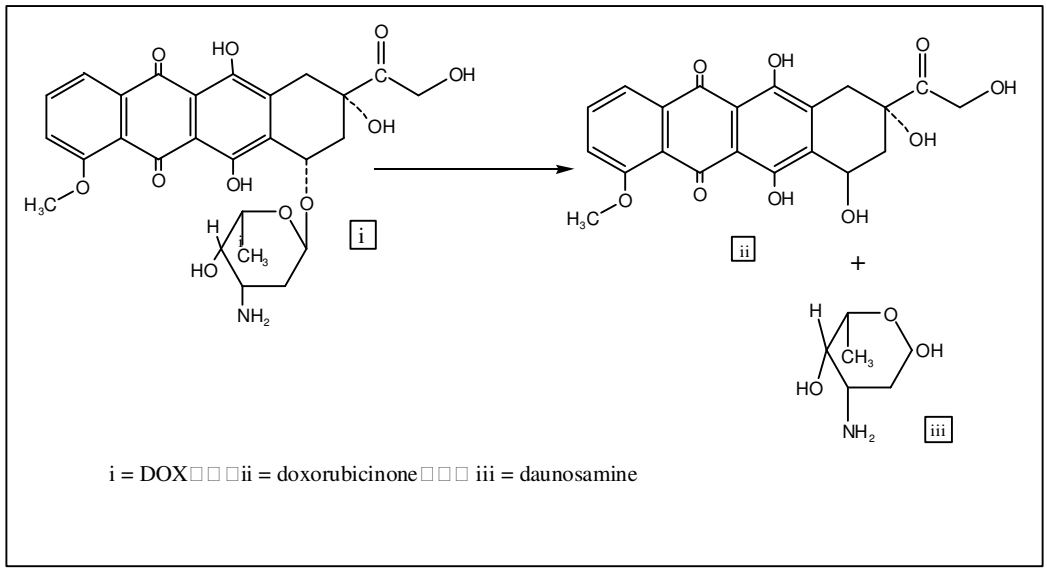
Figure 32. Scheme Showing the Hydrolysis of Cyclophosphamide at Neutral or Alkaline pH[5]



CP is temperature sensitive and hydrolysis may occur rapidly at temperatures above 30°C. The disappearance of the CP parent peak indicates degradation of CP, but further work would be needed to identify degradation products and assess any contribution to cytotoxicity.

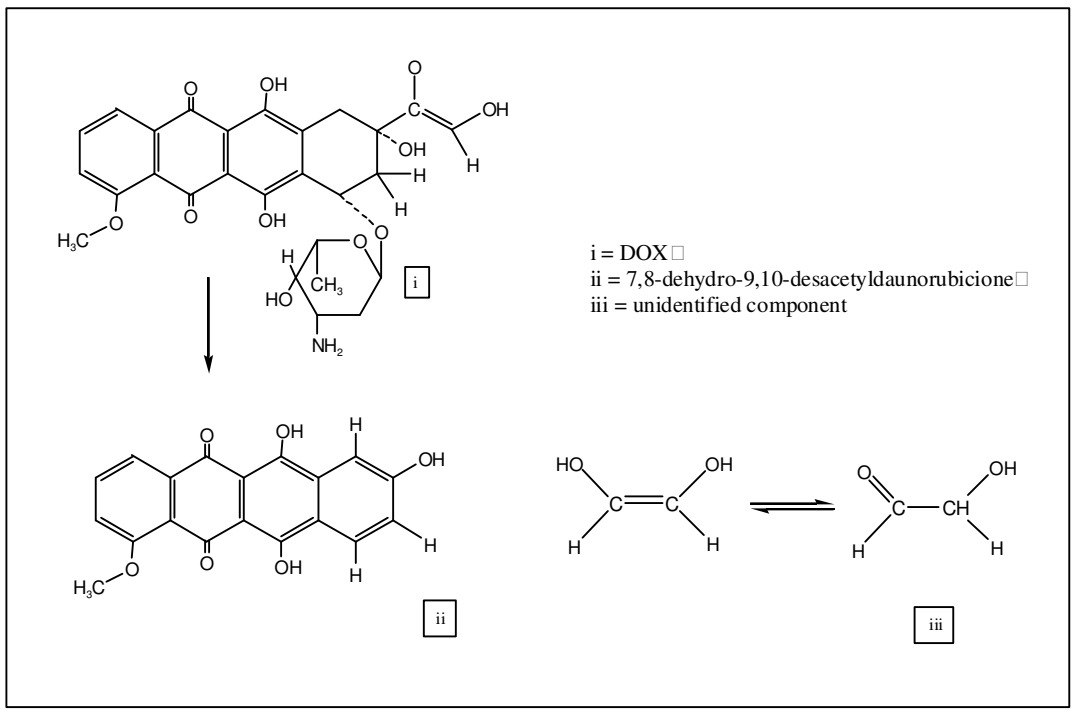
DOX and EPI have a similar stability profile. They exhibit pH-dependent stability in solution, with maximum stability observed at pH 4, and are sensitive to light and temperature.[24] The acid hydrolysis of DOX is shown schematically in Figure 33 below.[171] In solutions of pH less than 4, the glycosidic bond is cleaved and both release a water-insoluble tetracyclic aglycone (ii. doxorubicinone) and a red water-soluble amino sugar. The amino sugar released by DOX is daunosamine (iii)[171] and EPI releases acosamine.[24] The aglycone is cytotoxic but less so than the parent drug.[128]

Figure 33. Scheme Showing the Hydrolysis of Doxorubicin at Acidic pH[171]



In alkaline solution a colour change from red to deep purple is due to the rapid degradation of DOX and EPI.[24] This is thought to reflect cleavage of the amino sugar and the formation of other degradation products such as 7,8-dehydro-9,10 desacetyl-daunorubicnone.[153] The proposed reaction scheme is shown in Figure 34 below. The colour change also occurs with other anthracyclines antibiotics which are structurally similar.[128]

Figure 34. Scheme Showing the Degradation of Doxorubicin at Alkaline pH[153]



Mechanical removal by detergents was restricted to a small surface area where the contamination was visible. Scaling-up the procedure to remove surface contamination from an isolator would be more complex to undertake. The inside of an isolator covers a larger surface area of more complex topography. Some areas are not

accessible easily *via* the glove port system, making it difficult to wipe in a methodical way and apply pressure consistently over the whole area.

Exposure of the drugs to VHP® at different pH demonstrated the pH-dependent oxidation of DOX. Degradation between drugs was not comparable as the drugs demonstrate different stability profiles at various pH. For practical significance, further studies should explore cytotoxic decontamination on drugs exposed diluted in the standard pharmaceutical diluents only.

4.7 Conclusion

Mechanical removal by acidic, neutral and alkaline pH-based detergents was effective on 5-FU, CP and DOX surface contamination. The alkaline detergents, particularly CIP 100 which caused 81% degradation after 1 hour of contact with DOX, could be applied as part of a cleaning protocol for the removal and breakdown of DOX surface contamination. However, further studies would be required to identify the breakdown products and to establish whether these were still cytotoxic.

Two cleaning agents (Klerclean neutral detergent and Klercide CR-B) used currently in hospital pharmacies, IMS and WFI, were also effective in removing 5-FU, CP, DOX and EPI contamination. These cleaning agents have the potential of surface wipe cleaning only, as they were not effective in the breakdown of the cytotoxic drugs.

Exposure to VHP® at 1.6 $gmin^{-1}$ for 25 min caused the oxidative degradation of DOX. The effect was pH-dependent and the maximum amount of degradation from this exposure was 91.9%. 5-FU and CP were not susceptible to oxidation from VHP® under these conditions. A longer exposure time to VHP® for 2 hrs caused

degradation of CP, some degradation of 5-FU, and in addition caused the degradation of EPI.

VHP® is effective against microbial contamination. It also has the potential, with further investigation of cycle parameters to cause the degradation and maybe inactivation of surface cytotoxic contamination inside an isolator. However, to be applied successfully as a method for cytotoxic decontamination a guarantee would be required that the drug was completely degraded and that any degradation products would not contribute towards cytotoxicity. The breakdown products of 5-FU would not be expected to contribute any cytotoxicity, but CP, an oxasophosphorine prodrug which degrades to produce other alkylating cytotoxic compounds would need to be investigated thoroughly.

In conclusion, decontaminating an isolator using mechanical removal of cytotoxic residues with detergents is the method of most importance to hospital pharmacy practice. Using technology for chemical breakdown is less practical and would be more costly to implement into the daily running of a hospital pharmacy. If the products used for removal also cause chemical decomposition of the contamination, this could be potentially beneficial, depending on the extent of decomposition and the nature of the breakdown products

5. Comparative Contamination Study in an Isolator: traditional open-system versus a closed-system

5.1 Introduction

Although there have been comparative studies on effectiveness between the closed-system PhaSeal® device and traditional 'open' reconstitution methods,[67;78;124] no data have been reported on its effectiveness in an isolator under UK standards of practice. In the UK, drug reconstitution and administration is a pharmacy-based activity carried out in purpose designed facilities, which normally use isolators in preference to BSCs. Studies have been carried out in positive-pressure isolators under French standards of practice.[59;61] However, the need to investigate drug contamination found in pharmacies where negative-pressure isolators are normally used in the UK is apparent. In fact, apart from one recent study which carried out environmental monitoring in two UK hospital pharmacy units,[26] there appears to be a paucity of current UK data on occupational exposure.

Elimination of the primary contamination event would not only prevent potentially harmful exposure but would also prevent the occurrence of secondary contamination of areas outside the immediate drug environment.[20] Two systems for the compounding of cytotoxic drug injections/infusions were compared: the traditional 'open-system' using syringes, needles and venting needles, and a closed-system device, which is a special closed containment device for fluid-transfer.

5.1.1 The Traditional Open-System

The traditional open-system uses Luer-Lok syringes and needles, and hydrophobic filter needles as air vents. It is classified as an 'open-system' as it is well known that droplets can be liberated during manipulations using this method.[32;67]

5.1.2 The PhaSeal® Device - A Special Closed Containment Device

The PhaSeal® device prevents cytotoxic aerosols/droplets from contaminating the outside environment.[32] This was the closed-system device used during the Intervention period of this present study. The PhaSeal® system was an assembly of the following three main components;[127]

The Protector

The protector is a protective cover, which fits over the top of the vial (see Figure 35 below). It has a flexible expansion bulb which accommodates air pressure equalization when increased *i.e.* filling the vial with diluent, injecting air into the vial, or when a vacuum is created *i.e.* aspiration of a liquid. It contains a hydrophobic PTFE/polypropylene filter, which prevents vapour leakage.

Figure 35. Image of the Protector, and a Protector and Vial Assembled[127]

(reproduced with permission from Carmel Pharma)

The Injector

The injector (see Figure 36 on the following page) contains an encapsulated single lumen cannula, which locks into a disposable syringe *via* a Luer-Lok fitting. When the protector and injector are assembled, a double, tightly sealed, elastomeric

membrane is created. These membranes, made of elastomers are self-sealing and fit tightly together through a bayonet fitting which locks tightly with a twist. The cannula perforates the double membranes for the transfer of liquid, ensuring the tip of the cannula is never exposed, preventing needle-stick injuries. When the needle is retracted, the membranes seal off preventing leakage to the environment. This ensures a dry connection with only 'wet' retained behind each membrane. It also comprises a safety latch, which has to be released before the injector can be depressed.

Figure 36. Image of the Injector, and an Injector and Luer-Lok Syringe Assembled[127]

(reproduced with permission from Carmel Pharma)

The Infusion Adaptor

The infusion adaptor (see Figure 37 on the following page) connects *via* a puncture spike to the infusion bag. Transfer into the infusion bag from an Injector-fitted syringe is *via* a membrane-infused bayonet fitting on the infusion adaptor, allowing closed transfer into and out of the bag.

Figure 37. Image of the C71 Infusion Adaptor[127]

(reproduced with permission from Carmel Pharma)

A 2-arm cross-cohort study was designed to collect data during the compounding of cytotoxic drugs in an isolator. A cohort study was used to collect contamination data from the same surfaces, at a large number of sampling points, so that contamination produced from batch preparation could be assessed. An intense sampling schedule was applied to generate a large amount of data and assess the time sequence of events. A cross-over design was used to make individual comparisons between contamination measured on the same surfaces using the two different methods over the same period of time, one after another.

The compounding process was a simulated process and the test infusions produced were not administered to the patient. Baseline data were collected using existing standard practice (open-system) and intervention data were collected during implementation of a closed-system transfer (PhaSeal®) device. The effectiveness of intervention was investigated with two drugs novel to testing with the device; EPI and MTX, and also with CP. CP has been used frequently as a marker drug and the effectiveness of the closed-system (PhaSeal®) device in reducing CP contamination

when it was prepared in a BSC or on a bench top in the U.S and in continental Europe has been documented.[20;67;78;124] Methods developed and validated for these drugs as described in Chapter 3 (Method 2) were used to quantify the amount of contamination from surfaces and achieve the objectives as described below.

5.2 Study Objectives

The primary objectives were to investigate the following during the compounding of cytotoxic drugs in an isolator workstation:

i. determine the frequency and amount of contamination in an isolator workstation;
ii. determine if this contamination is persistent, or if it can be removed by cleaning;
iii. determine how much contamination is transferred to the outside environment *via* the external surface contamination of products leaving the isolator;
iv. determine the effectiveness of intervention (PhaSeal®) in reducing
    a. the frequency and level of this contamination in the isolator workstation and
    b. the frequency and level of surface contamination of finished syringe batches leaving the isolator.

The secondary objectives were to determine operator acceptance of using this intervention in the isolator setting.

5.3 Materials

Cytotoxic Drugs

Methotrexate 25 mg mL^{-1} Injection (lot R084426) was obtained from Mayne Pharma Plc, Leamington Spa, UK. Cyclophosphamide 500 mg Powder for Injection (lot 44139) was obtained from Pzifer Ltd, Kent, UK. Pharmorubicin Solution for

Injection 2.0 mg mL^{-1} (lot BF87B) was obtained from Pharmacia and Upjohn Ltd, Sandwich, UK.

Cleaning Agents

Klercide 70/30 sterile denatured ethanol (lot 051005EP), Klerclean sterile neutral detergent (lot 060203ND/4) and Klercide CR (lot 060310BP/4) sterile filtered biocide B were obtained from Shield Medicare, Surrey, UK.

Equipment

The closed-system (PhaSeal®) device components: protector 50 (lot 607217), injector N31 (lot 612705) and infusion adapter C100 (lot 509034) were supplied by Carmel Pharma, Gotëborg. Sweden. The negative-pressure isolator, ducted externally, model CDC-B (2-glove) was manufactured by Envair, Rossendale, UK.

Consumables

Luer-Lok Plastipak syringes, 3.0 mL (lot 04K03A), 10 mL (lot SC050314), 20 mL (lot SD060115), and 60 mL (lot SF050117), and microlance™ 3 syringe needles, 19G × 2" (lot 050416) were obtained from Becton Dickinson, Oxford, UK. Syringe tip caps (lot 6023D) and 0.2 µm hydrophobic filter needles, 20G (lot 181703) were obtained from Baxa Ltd, Berkshire, UK. Berner cytostatic protection gloves (lot 507452780), cytostatic protection gowns (lot 5914), cytotoxic workmats, BioClean 100 nitrile gloves, Stericlean® dry wipes (lot W016603) and Stericlean® prep pads (lot 529179) were obtained from Helapet Ltd, Bedfordshire, UK. Freeflex® 500 mL polyolefin intravenous infusion bags (lot MK72094) containing sodium chloride 0.9% were obtained from Baxter Healthcare Ltd, Berkshire, UK. Polypropylene 250 mL

containers and 15 mL polypropylene tubes were obtained from Sarstedt Ltd, Leics, UK.

5.4 Study Design

5.4.1 Pharmacy Staff and Equipment

Two technicians were involved in cytotoxic batch preparation. Technician One (a pharmacy technician) with 3 years experience of working with cytotoxic drugs in a hospital pharmacy, and Technician Two (a pharmacist), were trained in use of the closed-system (PhaSeal®) device over a period of two weeks.

The study was undertaken in a controlled GMP-compliant clean-room environment in an academic setting at the Department of Pharmacy, Kingston University, London. The environment in the clean-room was tested to EU grade Class B.[135] Work was carried out to simulate the compounding of cytotoxic drugs in one isolator by following the Standard Operating Procedures (see Appendix 5 for list of SOPs), according to current practice at Plymouth Hospitals NHS Trust, Derriford. This is an acute university hospital with a specialised MHRA licensed pharmacy aseptic unit producing in excess of 50,000 dose units of chemotherapy per year, shared between five isolator workstations. Throughout the study, standard safety precautions were adhered to and personal protective equipment was used.

The study was restricted to an isolator operating under negative-pressure, with air ducted to the external environment. It was designed with two glove ports and two interlocking hatches with a 2 min time delay. The selected isolator and the clean-room it was situated in were used only for the compounding of cytotoxic drugs for the purpose of this study. Neither the clean-room or the isolator had previous exposure to cytotoxic drugs prior to this study.

5.4.2 Work Schedule

The study was divided into 4 data collection periods, each of 1-week duration. Data were collected using the open-system to establish a Baseline (Baseline periods 1 and 2), and using the closed-system (PhaSeal®) device (Intervention periods 1 and 2). The open-system was the existing standard practice of the model study site (Plymouth). Baseline and Intervention data were collected over two one-week periods in each case. This included an initial one-week period for familiarisation with the closed-system (PhaSeal®) device as follows;

Week 1	Baseline 1
Week 2	Closed-system (PhaSeal®) device familiarisation
Week 3	Intervention 1
Week 4	Baseline 2
Week 5	Intervention 2

The study was conducted so that data were captured each day of a four consecutive day working week for the Baseline and the Intervention arms of the study. During the Intervention arm of the study, all manipulations were carried out using the closed-system (PhaSeal®) device. No data were collected during the familiarisation period.

5.4.3 Training of the Technicians

The technicians had two weeks of training prior to the start of the study, one with the open-system and one with the closed-system (PhaSeal®) device. The second week of the study was used as another training week with the device (closed-system device familiarisation).

A professional from Carmel Pharma, Gotëborg, Sweden, undertook training of the technicians and competency assessment with the closed-system (PhaSeal®) device. Both technicians achieved competence prior to implementation of the device. The technicians documented any leakages or spillages during all study weeks, and any problems, observations or difficulties during implementation with the closed-system (PhaSeal®) device.

The technicians rotated between batch preparation in the isolator and support work on a daily basis. Technician One prepared batches on days 1 and 3, and Technician Two prepared batches on days 2 and 4. This arrangement was the same for each study week. The technician who was not preparing batches on a particular day would act as the support, and would also carry out the Sampling Procedure, as described in Section 5.4.8. Both technicians were trained with the Sampling Procedure prior to the start of the study.

5.4.4 Test Drugs and Batch Production

EPI, MTX and CP were the cytotoxic drugs compounded. Table 31 on the following page shows details of the batches produced during the four-day week. Each day was divided into Session 1 and Session 2. In total, 25 batches of chemotherapy (201 dose units) were prepared on each four-day week. This was equivalent to a total throughput in one isolator of 10,452 dose units per year, representing 21% of the workload of the 'model' unit at Plymouth. The total amount of drug prepared weekly was made up of 370 mg of EPI (10.5%), 493mg of MTX (14.0%), and 2,650 mg of CP (75.4%). This workload was the same for each week of the study to remove bias in workload variation during the study. A log of each batch of chemotherapy prepared was produced, recording the date, infusion name, number of devices filled, volume of

fill, total amount of cytotoxic drug in each batch, type of container, time to prepare the batch and the name of the technician involved in batch production.

Table 31. Schedule of Batch Production of EPI, MTX and CP for Each Study Week

Day and Session	Drug	Volume (mL)	Infusion (mL)	Number of Units	Batch Number
Day 1, Session 1	CP, 150 mg	7.5	10.0 mL syringe	10	1
	CP, 500 mg	25.0	60.0 mL syringe	10	2
	EPI, 15.0 mg	7.5	10.0 mL syringe	15	3
Day 1, Session 2	EPI, 50.0 mg	25.0	60.0 mL syringe	10	4
	EPI, 75.0 mg	37.5	60.0 mL syringe	1	5
	MTX, 15.0 mg	0.6	3.0 mL syringe	10	6
	MTX, 200 mg	500	500 mL bag	1	7
Day 2, Session 1	CP, 200 mg	10.0	10.0 mL syringe	10	8
	EPI, 50.0 mg	25.0	50.0 mL syringe	10	10
	EPI, 40.0 mg	20.0	20.0 mL syringe	10	11
Day 2, Session 2	MTX, 20.0 mg	0.8	3.0 mL syringe	10	9
	MTX, 20.0 mg	0.8	3.0 mL syringe	10	12
	MTX, 40.0 mg	1.6	3.0 mL syringe	1	13
Day 3, Session 1	CP, 400 mg	20.0	20.0 mL syringe	10	14
	CP, 500 mg	25.0	60.0 mL syringe	10	15
	EPI, 20.0 mg	10.0	10.0 mL syringe	10	16
	EPI, 50.0 mg	25.0	60.0 mL syringe	10	17
Day 3, Session 2	MTX, 100 mg	500	500 mL bag	1	18
	MTX, 40.0 mg	1.6	3.0 mL syringe	1	19
Day 4, Session 1	CP, 400 mg	20.0	20.0 mL syringe	10	20
	CP, 500 mg	25.0	60.0 mL syringe	10	21
	EPI, 30.0 mg	15.0	20.0 mL syringe	10	22
	EPI, 40.0 mg	20.0	20.0 mL syringe	10	23
Day 4, Session 2	MTX, 17.5 mg	0.7	3.0 mL syringe	10	24
	MTX, 40.0 mg	1.6	3.0 mL syringe	1	25

5.4.5 Standards of Practice

The Plymouth Hospitals Standard Operating Procedures followed included use of the isolator (SOP CH7) and glove changing (SOP CH8), reconstitution of

cytotoxics and preparation of cytotoxic syringes (SOPs ASG8, ASG17, and CH1), dealing with cytotoxic spillages (SOP CH9), entering the isolator room (SOP CG6) and hatch transfer (SOP ASG2). The preparation of all batches was carried out in a similar manner. The following describes batch preparation on a day when Technician One would be preparing batches in the isolator.

Technician Two wiped all items with a wipe impregnated with IMS, and placed them into trays sprayed with IMS, prior to transfer into the clean-room. The technicians changed into their personal protective clothing *i.e.* overshoes, barrier hats, nitrile support gloves and chemotherapy gowns, and transferred items *via* the trolley transfer hatch into the clean-room. Technician Two gathered the appropriate supplies for the preparation of one batch at a time. The tray used to take items/consumables into the isolator though the right-hand hatch (when facing the isolator) was referred to as the 'tray in'. The 'tray in' was sprayed with IMS and the items sprayed into the tray. The outer door of the right hatch was opened and the 'tray in' placed inside. Technician One was seated and placed each arm and hand into the isolator sleeves and gloves. After 2 minutes, the right inner hatch door was opened, contents of the tray were removed and placed into the isolator main chamber. Prior to, during, and after the preparation of each batch, the isolator gloves were sprayed with IMS. When batch production was complete, the finished product was placed into a plastic bag in the left hatch. A different tray referred to as the 'tray out' was used to take any waste/items to be removed from the isolator *via* the left-hand hatch. The bag containing the finished product was removed from the left hatch *via* the outer door alongside the 'tray out'. Plastic backed absorbent mats were used for all preparations and changed at the end of each session, alongside with the gloves used in the isolator.

At the end of the session, Technician One cleaned the isolator and Technician Two carried out the Sampling Procedure as described in Section 5.4.8.

5.4.6 Cleaning Procedure

The isolator was cleaned twice daily. Prior to the start of Session 1 of each day, the right hatch was sprayed with Klercide CR-B (a blend of stabilized chlorine dioxide and a QAC), left for 5 min then wiped clean with IMS and a low lint swab. The procedure was repeated for the left hatch. The cleaning agents were transferred into the isolator and all accessible surfaces of the main chamber, including the sleeves were cleaned using the same method. All surfaces were then sprayed with IMS. Prior to the start of Session 2, the process was repeated except Klerclean neutral detergent (a non-ionic fatty alcohol ethoxylate), was used in place of Klercide CR-B. The tray was cleaned after each batch by spraying the inner and outer surfaces using Klercide CR-B and wiping with a sterile low lint wipe. At the end of the week, the floor was cleaned with distilled water and Klercide CR-B. A log was maintained of the cleaning procedures used and the time/date of cleaning.

5.4.7 Sampling Strategy

Sampling involved the removal of drug contamination from the specified areas of each surface, and was undertaken on four occasions (***a, b, c*** and ***d***) daily.

Sampling ***a*** was carried out at the beginning of each day prior to the start of Session 1 and loading of the isolator;

Sampling ***b*** was carried out at the end of Session 1, prior to cleaning;

Sampling ***c*** was carried out at the end Session 1 after cleaning, and prior to the start of Session 2 and loading of the isolator;

Sampling ***d*** was carried out at the end of Session 2 prior to the end of session cleaning.

This is outlined in the scheme below;

i.e. Day 1 sampling ***a***

Session 1 (batch preparation)

sampling ***b***

cleaning

sampling ***c***

Session 2 (batch preparation)

sampling ***d***

cleaning

Day 2 sampling ***a*** *etc*

Sampling was also carried out prior to the start of the study and before any cytotoxic drugs had entered the room or isolator.

5.4.8 Sampling Procedure

Each sampling time point (***a-d)*** involved wiping/swabbing predefined surfaces by wiping with an 70% IPA-impregnated wipe (Stericlean® prep pad). Validated methods, as described in Section 3.7.3 were used to wipe and remove the multi-drug contamination of EPI, MTX and CP from eleven surfaces. The contaminated wipes were collected and retained into 15 mL centrifuge (collection) tubes containing desorbing solution (3.5 mL) *i.e.* phosphate buffer (0.01 M) pH 4.0. Gloves used in the isolator (isolator gloves) and gloves used outside the isolator (support gloves) were

retained at the end of each session. The following areas were swabbed at each sampling point;

Isolator Base

Swabs were taken from three spot areas on the isolator base (see Section 3.7.1). A template measuring 21 cm × 21 cm was placed on the stainless steel surface and the swabs were taken within this area. The top left-hand side, top right-hand side, and the top centre were swabbed, using one wipe for each. The three-swabbed areas, in combination covered 21% of the area of the isolator base. All three swabs were combined in one collection tube.

Isolator Hatch Door

The hatch on the right-hand side of the isolator was used for the introduction of consumables into the isolator, and the hatch on the left was used for removal from the isolator. Each hatch door was made of a clear Perspex™ screen surrounded by a black rubber seal. Both sides of the screen of the right and left door leading directly into the isolator were swabbed *i.e.* the inner side of the hatch door in contact with the isolator (see Figure 38 on the following page); and the outer side of the door in contact with the hatch (see Figure 39 on the following page).

Isolator Sleeves

The isolator sleeves were made of Hypalon®. The cuffs of both the left and right sleeves were swabbed.

Figure 38. Image of the Isolator Base and Inner Hatch Doors

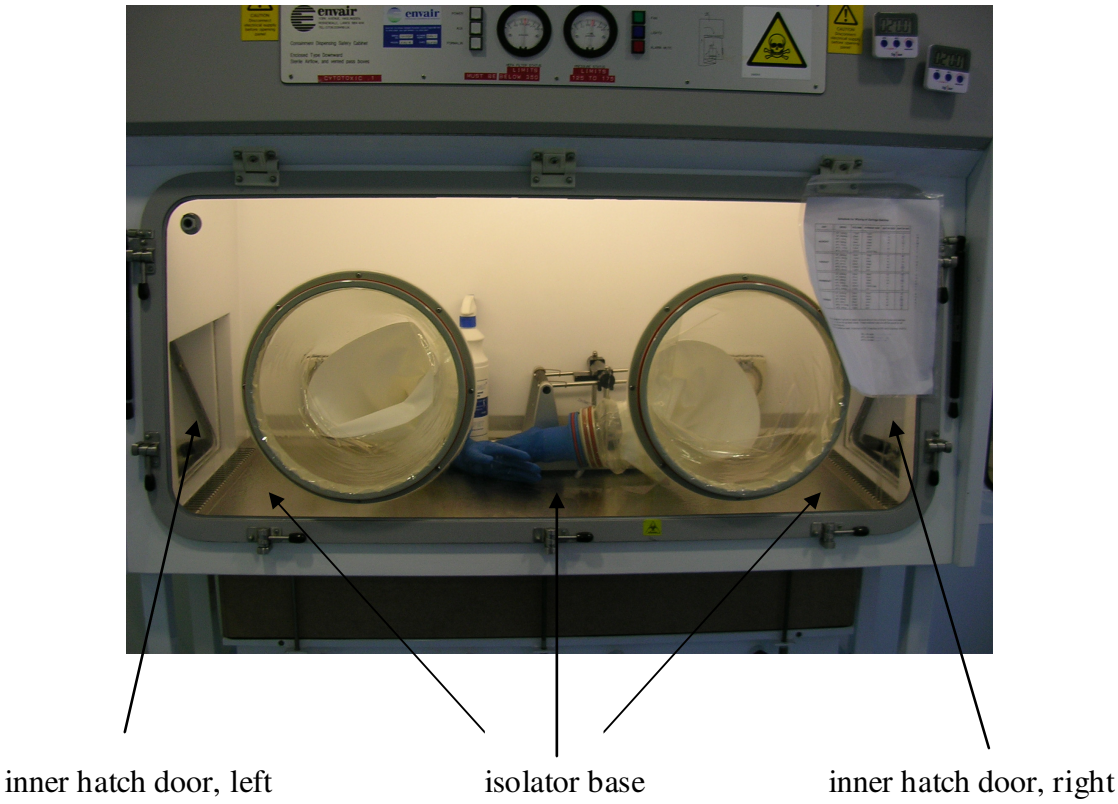
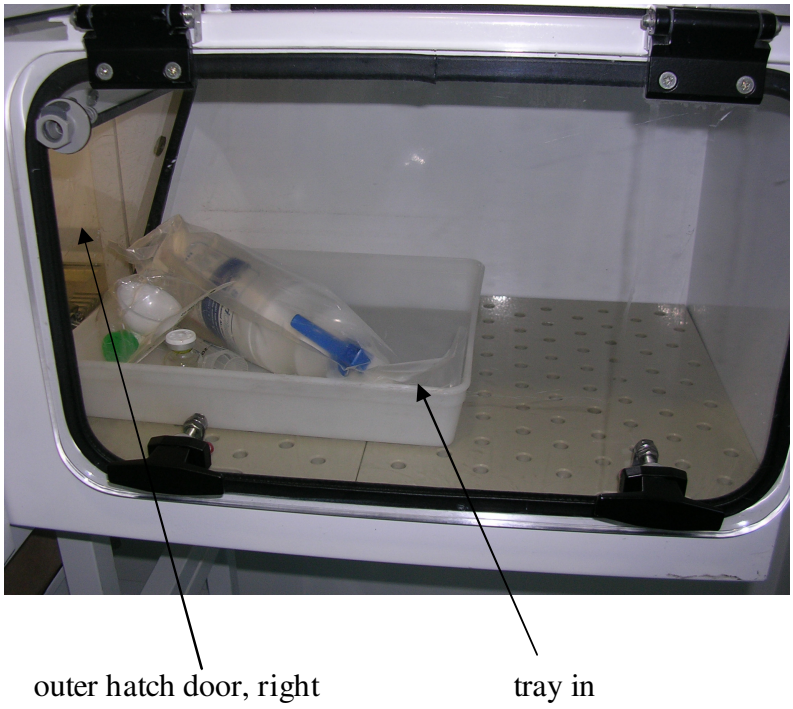


Figure 39. Image of the Right-Hand Outer Hatch Door and 'Tray In'



Tray

The same type and size of tray was used as the 'tray in' (see Figure 39 on the previous page) and the 'tray out'. The inside surface of both of the trays was swabbed.

Clean-room Floor

A square area was marked out on the blue vinyl floor of the clean-room, directly in front of the isolator and behind where the operator was positioned (see Figure 40 below). The marked area of the floor covered 21 cm × 21 cm. Special care was taken not to walk in this area during the study.

Figure 40. Image of the Marked Out Floor Area in Relation to the Isolator Workstation

Finished Product (syringes)

One third of the syringe batches produced were swabbed *i.e.* eight of the twenty-five batches prepared per week. In each case, these were all batches containing ten syringes, and all syringes from the batch were swabbed. The swabbed syringes were predetermined to be from batches 2, 4, 8, 12, 14, 16, 22, and 24 (three batches of EPI, three batches of CP, and two batches of MTX) - Table 31 (page 197). The syringe barrel and the plunger of the syringe were swabbed separately – see Figure 26 (page 121), using one wipe for each. The two wipes were combined into one collection tube.

5.4.9 Treatment of Samples

The contaminated wipe samples were placed into collection tubes, which were secured tightly and shaken to move the wipe down to be exposed to the desorbing solution.

The isolator gloves and support gloves were retained when they were changed at the end of each session. Each glove was removed inside out, carefully so as not to contaminate one with the other, and placed into a pre-labelled grip-top bag. The air was squashed out of the bag and it was sealed tightly.

The collection tubes containing the wipes and the bags containing the gloves were stored at -21°C. When due for transfer they were packed in dry ice and transported to the University of Bath where they remained in a temperature monitored laboratory freezer at -21°C until recovery and analysis.

5.4.10 Analysis of Samples

A validated method, as described in Section 3.7.3 to recover the multi-drug surface contamination of EPI, MTX and CP from the wipes into a desorbing solution

was applied. A validated immersion method, as described in Section 3.7.3 was applied to recover multi-drug contamination of EPI, MTX and CP from the isolator gloves and the support gloves into a desorbing solution.

The performance of the analytical method was monitored throughout by the analysis of freshly prepared quality control samples for each drug. Also, quality control samples which had been transported and stored alongside the surface contamination samples, carried through the recovery process and subjected to the same laboratory conditions *i.e.* temperature and time waiting in the autosampler prior to analysis, were analysed together with the surface contamination samples.

Levels of contamination of each drug were quantified in both the Baseline and Intervention arms of the study using HPLC methods, as described in Section 3.7.3. Responses below the LoQ of the analytical method (see Section 3.8.4) were reported as being present but not quantifiable *i.e.* <LoQ. Response below the LoD of the method was stated as 'not detected' *i.e.* ND.

5.4.11 Statistical Evaluation

Levels of contamination and site-specific frequencies were compared for each test drug in both arms of the study. Significance was established using descriptive statistical methods. Potential variables of contamination data for each system were explored.

5.4.12 Acceptance of the Closed-System (PhaSeal®) Device – A Questionnaire

Acceptance of the closed-system (PhaSeal®) device in the isolator setting was determined by questionnaire. The questionnaire comprised three parts (see Appendix 6 - Questionnaire to Determine Operator' Opinion on the Use of the Closed-System (PhaSeal®) Device). This was designed after literature review, consultation with a

professional from the manufacturers (Carmel Pharma), and a pharmacy manager of an ASU. It was piloted in the commercial ASU, Department of Pharmacy, University of Bath, by pharmacy technicians, to check the relevance and clarity of the questions.

Part 1 was administered to the technicians and completed prior to start of the study and training with the closed-system (PhaSeal®) device. It consisted of three closed questions, with the option of including comments, to learn from the experience of the technicians and how worried or safe they felt about working with cytotoxic drugs.

Part 2 was administered and completed by the technicians after training with the closed-system (PhaSeal®) device and prior to the start of the Intervention 1. It consisted of four questions. Three closed questions asked to select from a list how adequate the training they had been given with the closed-system (PhaSeal®) device was and how confident they felt using the device and that it was protecting them. A fourth open question asked to describe any reservations they might have with using the closed-system (PhaSeal®) device.

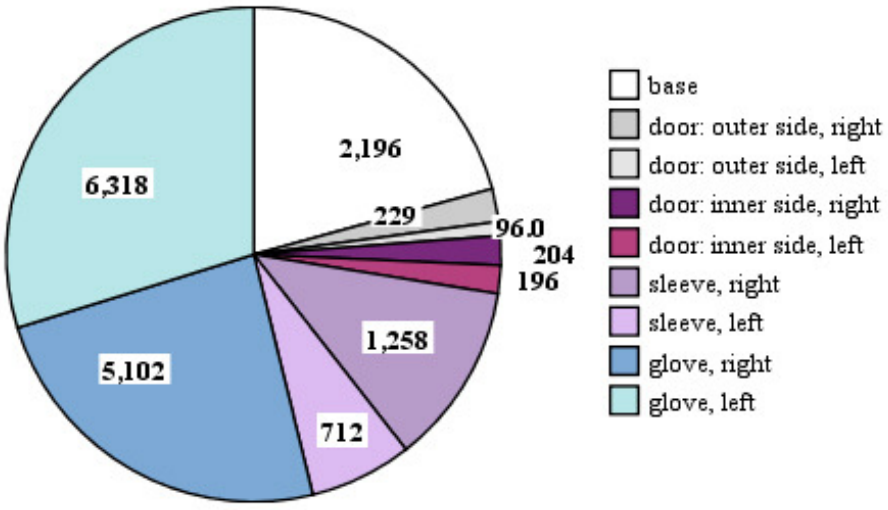
Part 3 was administered and completed by the technicians at the end of Intervention 2. It consisted of four questions. Two closed questions asked to select from a list how confident the technician was using the closed-system (PhaSeal®) device and if the same amount of care was applied regardless of the method. Two open questions asked the technicians to describe any problems encountered when using the device and if it hindered work in any way.

5.5 Results - Data Analysis and Interpretation

5.5.1 Surface Contamination Recovered from the Isolator

Over the duration of the four-week study, 960 wipe samples, 64 isolator gloves and 64 support gloves were collected. Prior to the start of the study, all wipe samples were negative for cytotoxic contamination. Therefore, the amount recovered during Baseline 1 can be attributed only to the batch production during this week - see Table 31 (page 197). A total amount of EPI (16,311 ng), MTX (10,988 ng) and CP (180,000 ng) were recovered from surfaces inside the isolator, cumulative of all the sampling points during Baseline 1. Figures 41, 42 and 43 (below and on the following page) show the amount of EPI, MTX and CP contamination, respectively, recovered from the isolator surfaces after one week of production. The results are shown in a pie chart for each drug, and are expressed in nanograms or nanograms per isolator glove or sleeve, cumulative of the week. Each coloured portion represents an area from where contamination was recovered.

Figure 41. Amount of EPI (ng, or ng per sleeve/glove) Recovered from Isolator Surfaces (Baseline 1)



EPI was recovered from all surfaces inside the isolator, with the highest amounts found on the isolator gloves and the base. MTX was recovered from the isolator base, gloves, left sleeve and both sides of the right-hand hatch door. CP was only recovered from the hatch door, both sides in contact with the hatch.

Figure 42. Amount of MTX (ng, or ng per sleeve/glove) Recovered from Isolator Surfaces (Baseline 1)

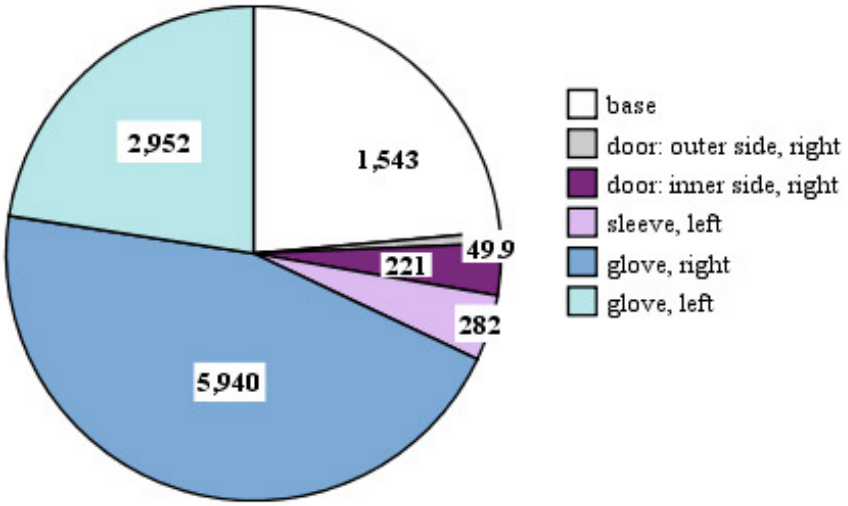
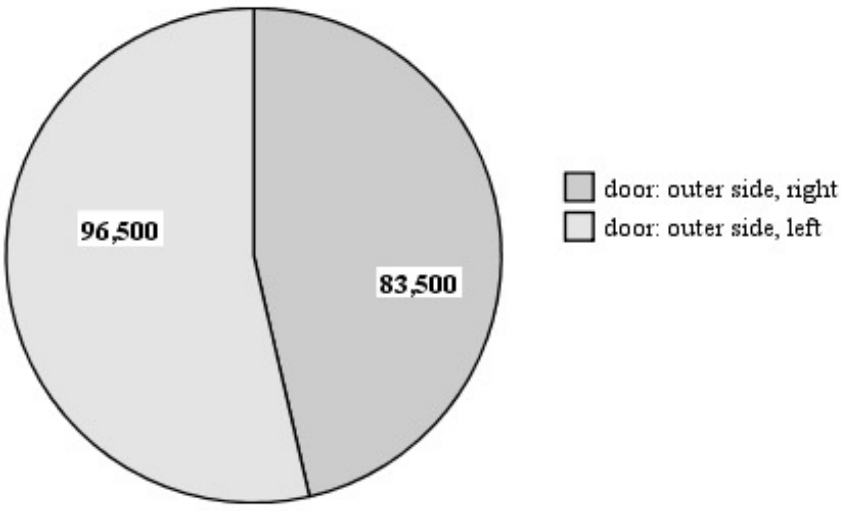


Figure 43. Amount of CP (ng) Recovered from Isolator Surfaces (Baseline 1)



This surface contamination recovered from the isolator during Baseline 1 was from the compounding of EPI (370 mg), MTX (493 mg) and CP (2,650 mg) - Table 31, page 197. Each mg of EPI, MTX and CP prepared contributed 44.1 ng, 22.3 ng and 67.9 ng of contamination, respectively. The mass of drug recovered (nanograms) directly after the corresponding session, and prior to any cleaning was normalised per mg of drug prepared in the session. The results of EPI and MTX recovered from areas inside the isolator per mg prepared during Baseline 1 are expressed in Table 32 below, and Table 33 on the following page, respectively.

Table 32. Amount of EPI Contamination Recovered (Baseline 1)

	Amount of EPI Recovered (ng per mg prepared)				
Area	**Day 1**	**1** (Session 2)	**2**	**3**	**4**
Isolator base	51.6	1.12	ND	1.40	0.76
Door: outer right	3.32	0.31	ND	ND	ND
Door: outer left	0.70	0.31	ND	ND	ND
Door: inner right	4.07	0.43	0.48	ND	ND
Door: inner left	3.80	0.40	0.55	ND	ND
Sleeve, right	47.4	0.72	0.46	0.61	ND
Sleeve, left	26.5	0.65	ND	ND	ND
Isolator glove, right	32.5	2.93	3.12	10.7	1.71
Isolator glove, left	28.6	3.35	3.53	7.81	1.56
Total	199	10.2	8.14	20.5	4.03

ND = none detected

EPI was only prepared in the morning session (Session 1) during the week, except on day 1 when two of the three batches were prepared in the afternoon session (Session 2). Batch preparation on Day 1, during Session 1 contributed the most EPI contamination to surfaces in the isolator per mg prepared. Batch 3 was the only batch of EPI prepared during this session therefore, the contamination recovered could be attributed only to this batch. The base and the right sleeve were the most highly

contaminated from the preparation of this batch. In total, on Day 1, 199 ng per mg of EPI prepared was recovered, a significant amount compared to sessions on other days.

Table 33. Amount of MTX Contamination Recovered (Baseline 1)

Area	Day	Amount of MTX Recovered (ng per mg prepared) 1	2	3	4
Isolator base		ND	ND	1.75	2.05
Door: outer right		ND	ND	ND	ND
Door: outer left		ND	ND	ND	ND
Door: inner right		ND	ND	ND	2.65
Door: inner left		ND	ND	ND	ND
Sleeve, right		ND	ND	ND	ND
Sleeve, left		ND	ND	ND	ND
Isolator glove, right		1.74	8.92	5.57	13.1
Isolator glove, left		1.99	4.98	0.48	2.60
Total		3.73	13.9	7.80	20.4

ND = none detected

MTX was prepared on each day but always during Session 2. MTX was recovered from the isolator gloves on every day of the working week and from the isolator base on days 3 and 4. Batches (24 and 25) prepared on day 4 (Table 31, page 197) contributed the most contamination per mg prepared.

CP was only prepared during Session 1. No CP was recovered from samples taken directly after batch preparation for the corresponding session during Baseline 1, although CP was recovered after sessions in which it was not prepared. Therefore, the results in Table 34 on the following page are expressed as the amount of CP recovered from areas in the isolator per mg prepared during Baseline 2. No CP was recovered from batches prepared during Day 1, 2 or 4 during Baseline 2.

Table 34. Amount of CP Contamination Recovered (Baseline 2)

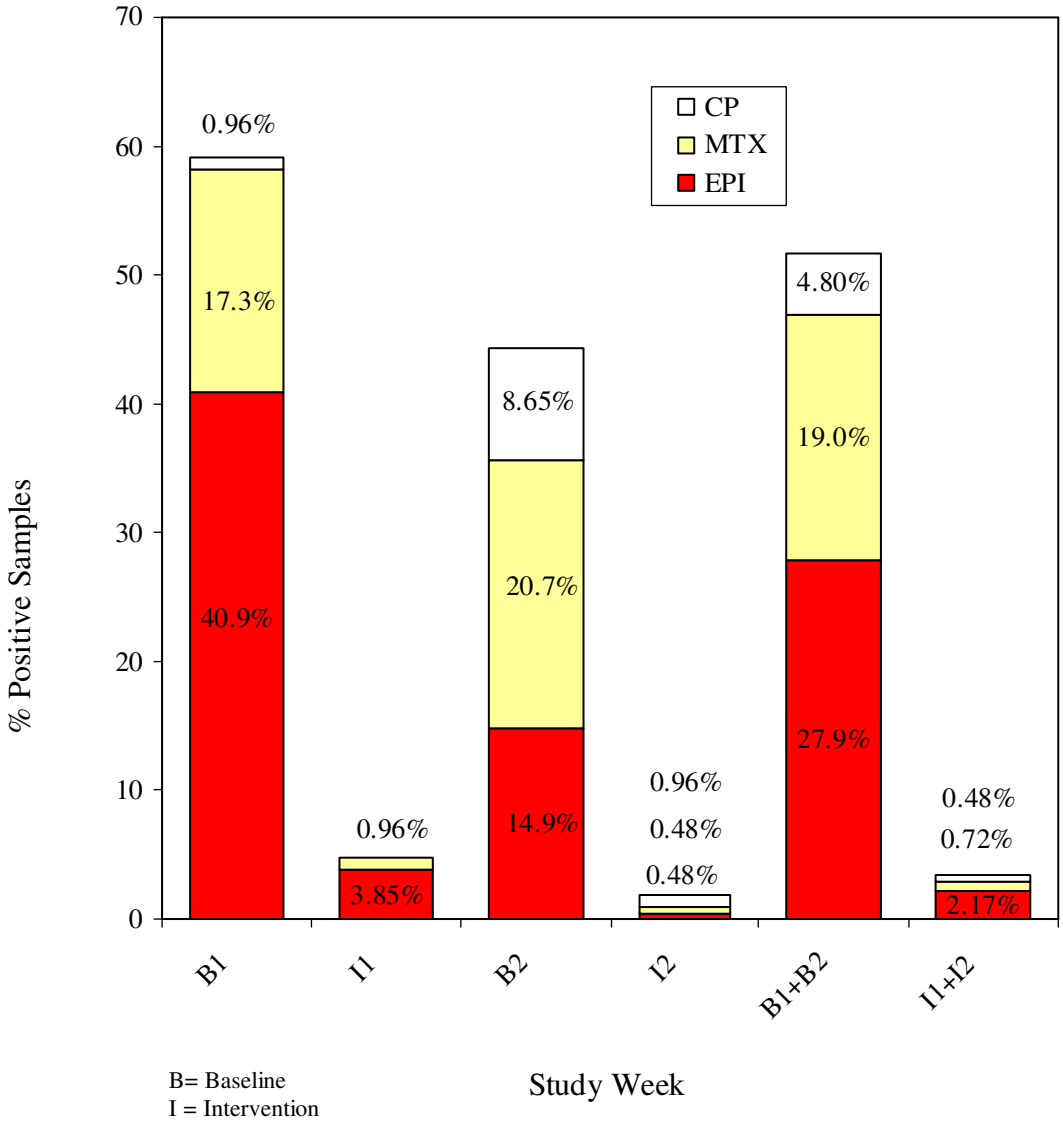
Area	Day	Amount of CP Recovered (ng per mg prepared) 1	2	3	4
Isolator base		ND	ND	ND	ND
Door: outer right		ND	ND	213	ND
Door: outer left		ND	ND	151	ND
Door: inner right		ND	ND	239	ND
Door: inner left		ND	ND	188	ND
Sleeve, right		ND	ND	338	ND
Sleeve, left		ND	ND	308	ND
Isolator glove, right		ND	ND	ND	ND
Isolator glove, left		ND	ND	ND	ND
Total		ND	ND	1,436	ND

ND = none detected

5.5.2 Effectiveness of the Closed-System (PhaSeal®) Device in Reducing Isolator Surface Contamination

The percentage of positive samples recovered for each drug during the Baseline (B1 and B2) weeks and Intervention (I1 and I2) weeks of the study are shown graphically in Figure 44 on the following page.

Figure 44. Graph Showing the Percentage of Positive Samples (EPI, MTX and CP) Recovered during the Baseline and Intervention Periods



The effect of the closed-system (PhaSeal®) device in reducing contamination in the isolator was investigated by comparing sampling data from Baseline and Intervention. Table 35 (Baseline 1 and 2), Table 36 (Intervention 1 and 2), and Table 37 (Baseline 1 + 2 combined, and Intervention 1 + 2 combined) on the following pages show the amount of contamination (median and range) and the frequency of positive samples recovered from all surfaces in the isolator during all weeks of the

study. All data have been normalised per square centimetre of the area sampled, except for gloves and sleeves, which are in ng or µg per isolator glove or sleeve. The LoD is also expressed in the same units, as the surfaces sampled from, varied in size.

Table 35. Surface and Glove Contamination Recovered from the Isolator during Baseline 1 and 2

Area	**Median**, Range and *Frequency* of Positive Samples above the LoD					
	Baseline 1			**Baseline 2**		
	median	range	*frequency*	**median**	range	*frequency*
Isolator base, ng cm^{-2}						
EPI	**0.06**	<0.002 - 0.59	*16/16*	**ND**	ND - <0.002	*3/16*
MTX	**ND**	ND - 0.33	*9/16*	**ND**	ND - 0.60	*4/16*
CP	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
Door: outer right, ng cm^{-2}						
EPI	**ND**	ND - 0.07	*6/16*	**ND**	ND - <0.003	*2/16*
MTX	**ND**	ND - 0.07	*3/16*	**ND**	ND - 0.34	*7/16*
CP	**ND**	ND - 117	*1/16*	**ND**	ND - 426	*3/16*
Door: outer left, ng cm^{-2}						
EPI	**ND**	ND - 0.07	*7/16*	**ND**	ND - <0.003	*2/16*
MTX	**ND**	ND - <0.07	*2/16*	**ND**	ND - 0.24	*7/16*
CP	**ND**	ND - 135	*1/16*	**ND**	ND - 393	*3/16*
Door: inner right, ng cm^{-2}						
EPI	**ND**	ND - 0.08	*8/16*	**ND**	ND - 0.05	*3/16*
MTX	**ND**	ND - 0.22	*3/16*	**ND**	ND - 8.04	*6/16*
CP	**ND**	all ND	*0/16*	**ND**	ND - 982	*3/16*
Door: inner left, ng cm^{-2}						
EPI	**ND**	ND - 0.08	*9/16*	**ND**	ND - <0.003	*5/16*
MTX	**ND**	ND - <0.07	*3/16*	**ND**	ND - 0.15	*5/16*
CP	**ND**	all ND	*0/16*	**ND**	ND - 2,034	*3/16*
Sleeve, right, ng per sleeve (EPI, MTX), µg per sleeve (CP)						
EPI	**41.5**	ND - 711	*11/16*	**ND**	ND - <2.0	*3/16*
MTX	**ND**	ND - <50.0	*1/16*	**ND**	ND - 216	*5/16*
CP	**ND**	all ND	*0/16*	**ND**	ND - 304	*2/16*
Sleeve, left, ng per sleeve (EPI, MTX), µg per sleeve (CP)						
EPI	**ND**	ND - 398	*12/16*	**ND**	ND - <2.0	*3/16*
MTX	**ND**	ND - 282	*5/16*	**ND**	ND - 716	*7/16*
CP	**ND**	all ND	*0/16*	**ND**	ND - 278	*3/16*
Isolator glove, right, ng per glove (EPI, MTX), µg per glove (CP)						
EPI	**486**	239 - 1,506	*8/8*	**217**	ND - 409	*5/8*
MTX	**653**	ND - 1,559	*6/8*	**ND**	ND - 748	*1/8*
CP	**ND**	all ND	*0/8*	**ND**	ND - 192	*1/8*
Isolator glove, left, ng per glove (EPI, MTX), µg per glove (CP)						
EPI	**737**	218 - 1,932	*8/8*	**206**	ND - 1,830	*5/8*
MTX	**226**	ND - 856	*6/8*	**ND**	ND - 750	*1/8*
CP	**ND**	all ND	*0/8*	**ND**	all ND	*0/8*

ND = none detected below the LoD
EPI LoD (ng cm^{-2}) = 0.001 (base, hatch door, tray), 0.002 (floor), 1.0 ng per sleeve/syringe, 2.0 ng per glove
MTX LoD (ng cm^{-2}) = 0.02 (base), 0.04 (hatch door, tray), 0.06 (floor), 25.0 ng per sleeve/syringe, 50.0 ng per glove
CP LoD (ng cm^{-2}) = 9.50 (base), 17.5 (hatch door), 18.0 (tray), 28.3 (floor), 12.5 µg per sleeve/syringe, 25.0 µg per glove

Table 36. Surface and Glove Contamination recovered from the Isolator during Intervention 1 and 2

Area	**Median**, Range and *Frequency* of Positive Samples above the LoD **Intervention 1**			**Intervention 2**		
	median	range	*frequency*	**median**	range	*frequency*
Isolator base, ng cm^{-2}						
EPI	**ND**	ND - <0.002	*1/16*	**ND**	all ND	*0/16*
MTX	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
CP	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
Door: outer right, ng cm^{-2}						
EPI	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
MTX	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
CP	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
Door: outer left, ng cm^{-2}						
EPI	**ND**	ND - 0.11	*2/16*	**ND**	all ND	*0/16*
MTX	**ND**	ND - <0.07	*1/16*	**ND**	all ND	*0/16*
CP	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
Door: inner right, ng cm^{-2}						
EPI	**ND**	ND - <0.003	*1/16*	**ND**	all ND	*0/16*
MTX	**ND**	ND - 0.07	*1/16*	**ND**	all ND	*0/16*
CP	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
Door: inner left, ng cm^{-2}						
EPI	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
MTX	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
CP	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
Sleeve, right, ng per sleeve (EPI, MTX), µg per sleeve (CP)						
EPI	**ND**	ND - 41.0	*1/16*	**ND**	all ND	*0/16*
MTX	**ND**	all ND	*0/16*	**ND**	ND - <50.0	*1/16*
CP	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
Sleeve, left, ng per sleeve (EPI, MTX), µg per sleeve (CP)						
EPI	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
MTX	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
CP	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
Isolator glove, right, ng per glove (EPI, MTX), µg per glove (CP)						
EPI	**ND**	ND - 171	*1/8*	**ND**	all ND	*0/8*
MTX	**ND**	all ND	*0/8*	**ND**	all ND	*0/8*
CP	**ND**	all ND	*0/8*	**ND**	ND - 138	*1/8*
Isolator glove, left, ng per glove (EPI, MTX), µg per glove (CP)						
EPI	**ND**	ND - 164	*2/8*	**ND**	ND - 161	*1/8*
MTX	**ND**	all ND	*0/8*	**ND**	all ND	*0/8*
CP	**ND**	all ND	*0/8*	**ND**	ND - 192	*1/8*

ND = none detected below the LoD
EPI LoD (ng cm^{-2}) = 0.001 (base, hatch door, tray), 0.002 (floor), 1.0 ng per sleeve/syringe, 2.0 ng per glove
MTX LoD (ng cm^{-2}) = 0.02 (base), 0.04 (hatch door, tray), 0.06 (floor), 25.0 ng per sleeve/syringe, 50.0 ng per glove
CP LoD (ng cm^{-2}) = 9.50 (base), 17.5 (hatch door), 18.0 (tray), 28.3 (floor), 12.5 µg per sleeve/syringe, 25.0 µg per glove

Table 37. Surface and Glove Contamination Recovered from the Isolator during Baseline 1 and 2 Combined, and Intervention 1 and 2 Combined

	Median, Range and *Frequency* of Positive Samples above the LoD					
Area	**Baseline 1 + 2**			**Intervention 1 + 2**		
	median	range	*frequency*	**median**	range	*frequency*
Isolator base, ng cm^{-2}						
EPI	**ND**	ND - 0.59	*19/32*	**ND**	ND - <0.002	*1/32*
MTX	**ND**	ND - 0.60	*13/32*	**ND**	all ND	*0/32*
CP	**ND**	all ND	*0/32*	**ND**	all ND	*0/32*
Door: outer right, ng cm^{-2}						
EPI	**ND**	ND - 0.07	*8/32*	**ND**	all ND	*0/32*
MTX	**ND**	ND - 0.34	*10/32*	**ND**	all ND	*0/32*
CP	**ND**	ND - 426	*4/32*	**ND**	all ND	*0/32*
Door: outer left, ng cm^{-2}						
EPI	**ND**	ND - 0.07	*9/32*	**ND**	ND - 0.11	*2/32*
MTX	**ND**	ND - 0.24	*9/32*	**ND**	ND - <0.07	*1/32*
CP	**ND**	ND - 393	*4/32*	**ND**	all ND	*0/32*
Door: inner right, ng cm^{-2}						
EPI	**ND**	ND - 0.08	*11/32*	**ND**	ND - <0.003	*1/32*
MTX	**ND**	ND - 8.04	*9/32*	**ND**	ND - 0.07	*1/32*
CP	**ND**	ND - 982	*3/32*	**ND**	all ND	*0/32*
Door: inner left, ng cm^{-2}						
EPI	**ND**	ND - 0.08	*14/32*	**ND**	all ND	*0/32*
MTX	**ND**	ND - 0.15	*8/32*	**ND**	all ND	*0/32*
CP	**ND**	ND - 2,034	*3/32*	**ND**	all ND	*0/32*
Sleeve, right, ng per sleeve (EPI, MTX), µg per sleeve (CP)						
EPI	**ND**	ND - 711	*14/32*	**ND**	ND - 41.0	*1/32*
MTX	**ND**	ND - 216	*6/32*	**ND**	ND - <50.0	*1/32*
CP	**ND**	ND - 304	*2/32*	**ND**	all ND	*0/32*
Sleeve, left, ng per sleeve (EPI, MTX), µg per sleeve (CP)						
EPI	**ND**	ND - 398	*15/32*	**ND**	all ND	*0/32*
MTX	**ND**	ND - 716	*12/32*	**ND**	all ND	*0/32*
CP	**ND**	ND - 278	*3/32*	**ND**	all ND	*0/32*
Isolator glove, right, ng per glove (EPI, MTX), µg per glove (CP)						
EPI	**272**	ND - 1,506	*13/16*	**ND**	ND - 171	*1/16*
MTX	**ND**	ND - 1,559	*7/16*	**ND**	all ND	*0/16*
CP	**ND**	ND - 192	*1/16*	**ND**	ND - 138	*1/16*
Isolator glove, left, ng per glove (EPI, MTX), µg per glove (CP)						
EPI	**737**	ND - 1,932	*13/16*	**ND**	ND - 164	*3/16*
MTX	**ND**	ND - 856	*7/16*	**ND**	all ND	*0/16*
CP	**ND**	all ND	*0/16*	**ND**	ND - 192	*1/16*

ND = none detected below the LoD
EPI LoD (ng cm^{-2}) = 0.001 (base, hatch door, tray), 0.002 (floor), 1.0 ng per sleeve/syringe, 2.0 ng per glove
MTX LoD (ng cm^{-2}) = 0.02 (base), 0.04 (hatch door, tray), 0.06 (floor), 25.0 ng per sleeve/syringe, 50.0 ng per glove
CP LoD (ng cm^{-2}) = 9.50 (base), 17.5 (hatch door), 18.0 (tray), 28.3 (floor), 12.5 µg per sleeve/syringe, 25.0 µg per glove

The complete tables of results showing the amount of drug recovered at each sampling point during the study period are shown in Appendices 7 (EPI), 8 (MTX) and 9 (CP).

Surface contamination data (ng cm^{-2}, or ng or µg per glove/sleeve) for the Baseline and Intervention methods were not normally distributed. Therefore, to compare the data using the two different methods, a non-parametric test; the Wilcoxon matched-pairs, signed ranks test (two-tailed) was used. The significance level of the test was $p \leq 0.05$, which meant there was sufficient evidence to reject the null hypothesis. The hypothesis were:

H_o – intervention with the closed-system (PhaSeal®) device does <u>not</u> reduce levels of cytotoxic surface contamination in the isolator,

H_1 – intervention with the closed-system (PhaSeal®) does reduce levels of cytotoxic surface contamination in the isolator.

Comparing Baseline 1 and Intervention 1 suggested significantly reduced levels of EPI and MTX surface contamination, (both $p = 0.001$) when the closed-system (PhaSeal®) device was implemented.

Comparing Baseline 2 and Intervention 2 suggested significantly reduced levels of EPI ($p = 0.003$), MTX ($p = 0.001$) and CP ($p = 0.002$) contamination when the closed-system (PhaSeal®) device was implemented.

There were an insufficient number of valid cases for comparison of CP contamination between Baseline 1 and Intervention 1. Combining data from Baseline 1 + 2, and Intervention 1 + 2 suggests significantly ($p = 0.001$) reduced levels of CP contamination when using the closed-system (PhaSeal®) device.

No problems were encountered with the closed-system (PhaSeal®) device, except on one occasion when a small spillage occurred while preparing EPI. The incident occurred on Day 2, during Session 1 of Intervention 1. The protector came away from the vial whilst separating the protector from the injector, spilling 3 drops of EPI onto the mat. The spillage was dealt with immediately; the syringe and vial were put into a plastic bag and transferred out of the isolator. The three remaining syringes from the batch were prepared away from the contamination on the mat. The isolator was cleaned down afterwards using the normal procedure. No surface contamination was recovered anywhere in the isolator after this session. Apart from this one incident, no leakages or spillages were reported which could have influenced levels of contamination during the study. There were also no recorded deviations from standard practice, so the levels of contamination, which were measured, were a result of the defined practice.

5.5.3 External Surface Contamination and Surface Cross-Contamination of Batches

It was evident that the external surfaces of batches prepared in the isolator are contaminated during the preparation process. The results of the median and range (ng or µg per syringe), and frequency of positive samples of EPI, MTX and CP contamination recovered from batches during Baseline 1 and 2, and Intervention 1 and 2 are expressed in Tables 38 – 43 on the following pages. Each batch was made up of ten syringes.

Table 38. EPI Contamination Recovered from External Surface of Syringe Batches during Baseline 1 and 2, and Baseline 1 and 2 Combined

Batch	**Median** and Range (ng per syringe), and *Frequency* of Positive Samples above the LoD								
	Baseline 1			**Baseline 2**			**Baseline 1 + 2**		
	median	range	*frequency*	**median**	range	*frequency*	**median**	range	*frequency*
EPI batches:									
4	**49.5**	42.0 - 78.5	*10/10*	**ND**	ND - <2.0	*7/10*	**21.0**	ND - 78.5	*17/20*
16	**20 .0**	ND - 64.5	*9/10*	**ND**	all ND	*0/10*	**ND**	ND - 64.5	*9/20*
22	**51.3**	<2.0 - 99.0	*10/10*	**45.0**	40.5 - 1,807	*10/10*	**50.0**	<2.0 - 1,807	*20/20*
MTX batches:									
12	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
24	**ND**	ND - 696	*1/10*	**<2.0**	<2.0 - 40.5	*10/10*	**ND**	ND - 696	*11/20*
CP batches:									
2	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
8	**ND**	ND - 41.5	*6/10*	**ND**	ND - <2.0	*3/10*	**ND**	ND - 41.5	*9/20*
14	**52.3**	ND - 61.5	*9/10*	**ND**	all ND	*0/10*	**ND**	ND - 61.5	*9/20*

ND = none detected below the LoD
EPI LoD = 1.0 ng per syringe

Table 39. EPI Contamination Recovered from External Surface of Syringe Batches during Intervention 1 and 2, and Intervention 1 and 2 Combined

Batch	**Median** and Range (ng per syringe), and *Frequency* of Positive Samples above the LoD								
	Intervention 1			**Intervention 2**			**Intervention 1 + 2**		
	median	range	*frequency*	**median**	range	*frequency*	**median**	range	*frequency*
EPI batches:									
4	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
16	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
22	**ND**	all ND	*0/10*	**ND**	ND - <2.0	*1/10*	**ND**	ND - <2.0	*1/20*
MTX batches:									
12	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
24	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
CP batches:									
2	**ND**	ND - <2.0	*2/10*	**ND**	all ND	*0/10*	**ND**	ND - <2.0	*2/20*
8	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
14	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*

ND = none detected below the LoD
EPI LoD = 1.0 ng per syringe

Table 40. MTX Contamination Recovered from External Surface of Syringe Batches during Baseline 1 and 2, and Baseline 1 and 2 Combined

Batch	**Median** and Range (ng per syringe), and *Frequency* of Positive Samples above the LoD								
	Baseline 1			**Baseline 2**			**Baseline 1 + 2**		
	median	range	*frequency*	**median**	range	*frequency*	**median**	range	*frequency*
EPI batches:									
4	**ND**	ND - 205	*1/10*	**ND**	all ND	*0/10*	**ND**	ND - 205	*1/20*
16	**ND**	all ND	*0/10*	**ND**	ND - <50.0	*3/10*	**ND**	ND - <50.0	*3/20*
22	**ND**	ND - <50.0	*1/10*	**ND**	ND - <50.0	*1/10*	**ND**	ND - <50.0	*2/20*
MTX batches									
12	**ND**	ND - 73.0	*1/10*	**ND**	all ND	*0/10*	**ND**	ND - 73.0	*1/20*
24	**ND**	ND - 259	*1/10*	**ND**	ND - <50.0	*3/10*	**ND**	ND - 259	*4/20*
CP batches:									
2	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
8	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
14	**ND**	ND - <50.0	*1/10*	**ND**	all ND	*0/10*	**ND**	ND - <50.0	*1/20*

ND = none detected below the LoD
MTX LoD = 25.0 ng per syringe

Table 41. MTX Contamination Recovered from External Surface of Syringe Batches during Intervention 1 and 2, and Intervention 1 and 2 Combined

Batch	**Median** and Range (ng per syringe), and *Frequency* of Positive Samples above the LoD								
	Intervention 1			**Intervention 2**			**Intervention 1 + 2**		
	median	range	*frequency*	**median**	range	*frequency*	**median**	range	*frequency*
EPI batches:									
4	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
16	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
22	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
MTX batches:									
12	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
24	**ND**	all ND	*0/10*	**ND**	ND - 81.5	*1/10*	**ND**	ND - 81.5	*1/20*
CP batches:									
2	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
8	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
14	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*

ND = none detected below the LoD
MTX LoD = 25.0 ng per syringe

Table 42. CP Contamination Recovered from External Surface of Syringe Batches during Baseline 1 and 2, and Baseline 1 and 2 Combined

Batch	**Median** and Range (µg per syringe), and *Frequency* of Positive Samples above the LoD								
	Baseline 1			**Baseline 2**			**Baseline 1 + 2**		
	median	range	*frequency*	**median**	range	*frequency*	**median**	range	*frequency*
EPI batches:									
4	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
16	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
22	**ND**	all ND	*0/10*	**ND**	ND - 75.0	*4/10*	**ND**	ND - 75.0	*4/20*
MTX batches:									
12	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
24	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
CP batches:									
2	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
8	**ND**	ND - 41.5	*2/10*	**ND**	all ND	*0/10*	**ND**	ND - 41.5	*2/20*
14	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*

ND = none detected below the LoD
CP LoD = 12.5 µg per syringe

Table 43. CP Contamination Recovered from External Surface of Syringe Batches during Intervention 1 and 2, and Intervention 1 and 2 Combined

Batch	**Median** and Range (µg per syringe), and *Frequency* of Positive Samples above the LoD								
	Intervention 1			**Intervention 2**			**Intervention 1 + 2**		
	median	range	*frequency*	**median**	range	*frequency*	**median**	range	*frequency*
EPI batches:									
4	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
16	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
22	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
MTX batches:									
12	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
24	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
CP batches:									
2	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
8	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*
14	**ND**	all ND	*0/10*	**ND**	all ND	*0/10*	**ND**	all ND	*0/20*

ND = none detected below the LoD
CP LoD = 12.5 µg per syringe

The complete tables of results showing the amount of drug recovered per syringe for each batch during the study period are shown in Appendices 7 (EPI), 8 (MTX) and 9 (CP).

EPI was recovered from all EPI batches during Baseline 1 (Table 38, page 219). A total of 29 of the 30 (96.7%), and 17 out of 30 (56.7%) of the syringes swabbed during Baseline 1 and 2, respectively, were positive for EPI contamination on the external surface of the syringe. The highest amount of EPI was 1,807 ng recovered from a syringe (batch 22) of the corresponding drug infusion during Baseline 2. No EPI contamination of the corresponding drug infusion was recovered during Intervention, except during Intervention 2, when trace was observed on one syringe from batch 22 (Table 39, page 220).

A total of 2 out of 20 (10%), and 3 out of 20 (15%) syringes swabbed during Baseline 1 and 2, respectively, which were filled with MTX, tested positive for MTX (Table 40, page 221). During Intervention, MTX contamination was only observed on one occasion when 81.5 ng was recovered from one syringe (batch 24) of the corresponding drug infusion during Intervention 2 (Table 41, page 222). Although this was a positive event, the amount recovered was less than the amount recovered from one syringe (259 ng) of the same batch prepared during Baseline 1.

The external contamination of CP on batches of CP was recovered less frequently. Of the 30 syringes, which were filled with CP during Baseline 1, 2 (6.7%) of the syringes swabbed tested positive for CP. The highest amount of CP was 41.5 µg recovered from a syringe (batch 8) of the corresponding drug infusion during Baseline 1 (Table 42, page 223). There were no positive cases of CP contamination on syringes of CP recovered during Baseline 2, Intervention 1 or 2 (Table 43, page 224).

It was evident that surface cross-contamination on the external surfaces of batches of syringes also occurs. Cross-contamination of EPI was recovered on 1 out of 20 (5%) syringes filled with MTX, and 15 out of 30 (50%) filled with CP during Baseline 1. The highest amount of EPI cross-contamination recovered was 696 ng on a single syringe of MTX (batch 24) and 61.5 ng on a batch of CP (batch 14), during Baseline 1. During Baseline 2, cross-contamination of EPI was recovered on 10 out of 20 (50%) syringes filled with MTX, and 3 out of 30 (10%) filled with CP (Table 38, page 219). No cross-contamination of EPI on batches of MTX was observed during Intervention 1 or 2, but trace amounts were recovered from two syringes of a batch of CP during Intervention 1 (Table 39, page 220).

Cross-contamination of MTX was recovered on 2 out of 30 (6.7%) of syringes filled with EPI, and 1 out of 30 (3.3%) filled with CP during Baseline 1. The largest amount of MTX cross-contamination was 205 ng recovered from a single syringe of EPI (batch 4) during Baseline 1. During Baseline 2, cross-contamination of MTX was recovered on 4 out of 30 (13.3%) syringes filled with EPI, and none filled with CP (Table 40, page 221). No cross-contamination of MTX on batches of EPI or CP was recovered during Intervention 1 or 2 (Table 41, page 222).

No cross-contamination of CP was observed on batches of EPI or MTX during Baseline 1 or on MTX batches during Baseline 2. Cross-contamination of CP on 4 out of 30 (13.3%) batches of EPI was recovered during Baseline 2. The highest amount of CP cross-contamination was 75.0 µg recovered from a syringe of EPI (batch 22) during Baseline 2 (Table 42, page 223). No CP cross-contamination on batches of EPI or MTX was recovered during Intervention 1 or 2 (Table 43, page 224).

5.5.4 Effectiveness of the Closed-System (PhaSeal®) Device in Reducing External Surface Contamination and Cross-Contamination of Batches

External surface contamination recovered from syringe batches (ng or µg per syringe) for the Baseline (1 + 2 combined) method versus the Intervention (1 + 2 combined) method was compared using the Wilcoxon matched-pairs, signed ranks test (two-tailed). The significance level of the test was $p \leq 0.05$, which meant there was sufficient evidence to reject the null hypothesis. The hypothesis were:

H_o – intervention with the closed-system (PhaSeal®) device does <u>not</u> reduce levels of external cytotoxic surface contamination on the batches of syringes,

H_1 – intervention with the closed-system (PhaSeal®) does reduce levels of external cytotoxic surface contamination on the batches of syringes.

The closed-system (PhaSeal®) device significantly reduced levels of EPI contamination ($p = 0.000$) on the outside surfaces of EPI batches, and on the surface of CP syringes ($p = 0.012$). The frequency of the occurrence of positive measurable cases of EPI cross-contamination on batches of MTX was insufficient for statistical comparison between the two techniques. However, implementation of the closed-system (PhaSeal®) device reduced the number of positive cases of EPI cross-contamination on syringes of MTX from 11 to 0 cases during the Baseline and Intervention periods, respectively.

The frequency of the occurrence of positive measurable cases of MTX contamination was insufficient to compare statistically between the two techniques in reducing contamination of MTX on batches of the corresponding drug infusion, and

cross-contamination on batches of EPI or CP. However, it was effective in reducing the number of positives cases of MTX contamination on syringes of the corresponding drug infusion from 5 to 1, on syringes of CP from 1 to 0, and 6 to 0 positive cases on syringes of EPI during the Baseline and Intervention periods, respectively.

There were an insufficient number of measurable positive cases to statistically assess the effectiveness of the closed-system (PhaSeal®) device in preventing the contamination of CP on batches of the corresponding drug infusion, and CP cross-contamination on batches of EPI and MTX. However, the closed-system (PhaSeal®) device was effective in reducing the number of positive cases of CP contamination on syringes of the corresponding drug infusion from 2 to 0, and the cross-contamination of CP on syringes of EPI from 4 to 0, during Baseline and Intervention, respectively. There were no positive cases of CP contamination on any syringes filled with MTX during the Baseline or Intervention periods.

The amount of contamination of the corresponding drug infusion recovered from the surfaces of syringes was normalised to explore relationships between the number of dose units prepared; the amount of drug prepared (mg) and the volume prepared (mL). The results of the calculations from Baseline 1 data are shown in Table 44 on the following page.

Batches 4 and 22 were the most contaminating per unit prepared to the exterior of syringes of the corresponding EPI batch. Batch 22 was the most contaminating per mg or per mL prepared. Batch 4 was prepared in 60 mL syringes, batch 16 in 10 mL syringes, and batch 22 in 20 mL syringes. Overall EPI, batch 22

prepared in 20 mL syringes appeared to be the most contaminated on the outer syringe surface.

Both batches (12 and 24) of MTX were prepared in 3.0 mL syringes. MTX, batch 24 was the most contaminating (approximately a 4-fold increase) per unit mass and volume prepared compared to batch 12.

Table 44. Amount External Syringe Contamination Recovered from Batches of the Corresponding Drug Infusion during Baseline 1, Normalised per Number of Units/Mass of Drug Prepared/Volume of Drug Prepared

	Amount of Contamination Recovered from Batches during Baseline 1		
Batch	ng per unit prepared	ng per mg prepared	ng per mL prepared
EPI, batch 4	53.2	10.6	2.13
EPI, batch 16	23.1	11.6	2.31
EPI, batch 22	47.9	16.0	3.19
MTX, batch 12	7.30	3.65	9.13
MTX, batch 24	25.9	14.8	37.0
CP, batch 2	ND	ND	ND
CP, batch 8	7,700	385	770
CP, batch 14	ND	ND	ND

ND = none detected

Of the three batches of CP prepared, contamination was only recovered on syringes from one batch (batch 8). CP was prepared in 60 mL syringes (batch 2), 10 mL syringes (batch 8) and 20 mL syringes (batch 14). Batch 8, prepared in the smallest syringe was the most contaminated compared to all the batches prepared during Baseline 1.

5.5.5 Contamination Recovered from Surfaces Outside the Isolator

Contamination was also recovered from areas outside the isolator *i.e.* the floor, the 'tray in' and 'tray out', and the support gloves. Tables 45, 46 and 47 on the

following pages show the amount of contamination (median and range) and the frequency of positive samples recovered outside the isolator during Baseline 1 and 2, Intervention 1 and 2, Baseline 1 + 2 combined, and Intervention 1 + 2 combined, respectively. The complete tables of results are shown in Appendices 7 (EPI), 8 (MTX) and 9 (CP).

Table 45. Surface Contamination Recovered from Surfaces Outside the Isolator during Baseline 1 and 2

	Median, Range and *Frequency* of Positive Samples above the LoD					
Area	**Baseline 1**			**Baseline 2**		
	median	range	*frequency*	**median**	range	*frequency*
Floor, ng cm^{-2}						
EPI	**ND**	ND - 0.11	*7/16*	**ND**	ND - <0.005	*1/16*
MTX	**ND**	ND - <0.11	*3/16*	**ND**	ND - 11.3	*4/16*
CP	**ND**	all ND	*0/16*	**ND**	ND - 128	*3/16*
Tray in, ng cm^{-2}						
EPI	**0.07**	ND - 0.19	*13/16*	**ND**	all ND	*0/16*
MTX	**ND**	ND - 0.19	*4/16*	**ND**	ND - 0.08	*3/16*
CP	**ND**	all ND	*0/16*	**ND**	ND - 384	*5/16*
Tray out, ng cm^{-2}						
EPI	**0.06**	ND - 0.07	*13/16*	**ND**	ND - <0.003	*3/16*
MTX	**ND**	ND - 0.21	*6/16*	**ND**	ND - <0.07	*2/16*
CP	**ND**	all ND	*0/16*	**ND**	ND - 137	*4/16*
Support glove, right, ng per glove (EPI, MTX), µg per glove (CP)						
EPI	**1,037**	165 - 12,823	*8/8*	**ND**	all ND	*0/8*
MTX	**ND**	all ND	*0/8*	**ND**	all ND	*0/8*
CP	**910**	ND - 1,216	*6/8*	**790**	97.6 - 1,111	*8/8*
Support glove, left, ng per glove (EPI, MTX), µg per glove (CP)						
EPI	**1,248**	178 - 13,268	*8/8*	**ND**	ND - 172	*2/8*
MTX	**ND**	all ND	*0/8*	**ND**	all ND	*0/8*
CP	**819**	ND - 1,356	*7/8*	**870**	612 - 946	*8/8*

ND = none detected
EPI LoD (ng cm^{-2}) = 0.002 (floor), 0.001 (tray), 2.0 ng per glove
MTX LoD (ng cm^{-2}) = 0.06 (floor), 0.04 (tray), 50.0 ng per glove
CP LoD (ng cm^{-2}) = 28.3 (floor), 18.0 (tray), 25.0 µg per glove

Table 46. Surface Contamination Recovered from Surfaces Outside the Isolator during Intervention 1 and 2

Area	**Median**, Range and *Frequency* of Positive Samples above the LoD					
	Intervention 1			**Intervention 2**		
	median	range	*frequency*	**median**	range	*frequency*
Floor, ng cm^{-2}						
EPI	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
MTX	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
CP	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
Tray in, ng cm^{-2}						
EPI	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
MTX	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
CP	**ND**	ND - 214	*3/16*	**ND**	ND - 91.2	*4/16*
Tray out, ng cm^{-2}						
EPI	**ND**	ND - <0.003	*1/16*	**ND**	all ND	*0/16*
MTX	**ND**	ND - 0.14	*1/16*	**ND**	ND - 0.39	*2/16*
CP	**ND**	ND - 183	*2/16*	**ND**	all ND	*0/16*
Support glove, right, ng per glove (EPI, MTX), µg per glove (CP)						
EPI	**ND**	ND - 189	*1/8*	**ND**	ND - 172	*2/8*
MTX	**ND**	all ND	*0/8*	**ND**	all ND	*0/8*
CP	**455**	96.0 - 1,146	*8/8*	**568**	46.0 - 865	*8/8*
Support glove, left, ng per glove (EPI, MTX), µg per glove (CP)						
EPI	**ND**	ND - 1,046	*2/8*	**ND**	ND - 177	*2/8*
MTX	**ND**	all ND	*0/8*	**ND**	all ND	*0/8*
CP	**590**	199 - 873	*8/8*	**588**	1,027 – 1,476	*8/8*

ND = none detected

EPI LoD (ng cm^{-2}) = 0.002 (floor), 0.001 (tray), 2.0 ng per glove

MTX LoD (ng cm^{-2}) = 0.06 (floor), 0.04 (tray), 50.0 ng per glove

CP LoD (ng cm^{-2}) = 28.3 (floor), 18.0 (tray), 25.0 µg per glove

Table 47. Surface Contamination Recovered from Surfaces Outside the Isolator during Baseline 1 and 2 Combined, and Intervention 1 and 2 Combined

Area	**Median**, Range and *Frequency* of Positive Samples above the LoD **Baseline 1 and 2 Combined**			**Intervention 1 and 2 Combined**		
	median	range	*frequency*	**median**	range	*frequency*
Floor, ng cm^{-2}						
EPI	**ND**	ND - 0.11	*8/32*	**ND**	all ND	*0/32*
MTX	**ND**	ND - 11.3	*7/32*	**ND**	all ND	*0/32*
CP	**ND**	ND - 128	*3/32*	**ND**	all ND	*0/32*
Tray in, ng cm^{-2}						
EPI	**ND**	ND - 0.19	*13/32*	**ND**	all ND	*0/32*
MTX	**ND**	ND - 0.19	*7/32*	**ND**	all ND	*0/32*
CP	**ND**	ND - 384	*5/32*	**ND**	ND - 214	*7/32*
Tray out, ng cm^{-2}						
EPI	**ND**	ND - 0.07	*16/32*	**ND**	all ND	*1/32*
MTX	**ND**	ND - 0.21	*8/32*	**ND**	ND - 0.39	*3/32*
CP	**ND**	ND - 137	*4/32*	**ND**	ND - 183	*2/32*
Support glove, right, ng per glove (EPI, MTX), µg per glove (CP)						
EPI	**1037**	ND - 12,823	*8/16*	**ND**	ND - 189	*3/16*
MTX	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
CP	**790**	400 - 1,216	*14/16*	**455**	46.0 - 1,146	*16/16*
Support glove, left, ng per glove (EPI, MTX), µg per glove (CP)						
EPI	**175**	ND - 13,268	*10/16*	**ND**	ND – 1,046	*4/16*
MTX	**ND**	all ND	*0/16*	**ND**	all ND	*0/16*
CP	**870**	ND - 1,356	*15/16*	**950**	199 - 1,476	*16/16*

ND = none detected
EPI LoD (ng cm^{-2}) = 0.002 (floor), 0.001 (tray), 2.0 ng per glove
MTX LoD (ng cm^{-2}) = 0.06 (floor), 0.04 (tray), 50.0 ng per glove
CP LoD (ng cm^{-2}) = 28.3 (floor), 18.0 (tray), 25.0 µg per glove

During the Baseline periods, EPI and CP were recovered from all surfaces outside the isolator. The largest amounts were recovered from the support gloves during Baseline 1 for EPI and during Baseline 2 for CP. Contamination of the support gloves with CP were consistently high during all weeks of the study, regardless of the system used. In fact, similar amounts of CP were recovered during the Intervention periods combined (1,476 µg) compared to the Baseline periods combined (1,356 µg) - Table 47. The trays 'in and out' were also found to be contaminated with similar

levels of CP during the Baseline periods compared to the Intervention periods. There appears to be a relationship between the 'tray in' and areas in the isolator testing positive for CP during Baseline 2, since contamination was recovered from the isolator only when the 'tray in' was also found to be contaminated. No MTX contamination was recovered from the support gloves during either the Baseline or Intervention periods, but it was present on the 'tray in and out' and on the floor during the Baseline periods.

During Intervention, there was no cytotoxic contamination of any of the three drugs recovered from the floor, and no EPI or MTX contamination was recovered from the 'tray in.' Contamination of all three drugs was recovered from the 'tray out' during the Intervention periods. More MTX was recovered from the 'tray out' during the Intervention periods than during the Baseline periods, however, the frequency of positive cases of contamination of all three drugs was less than during the Baseline periods.

5.5.6 Effectiveness of Cleaning in Removing Surface Contamination during Baseline

The amount of contamination recovered before and after cleaning the isolator during Baseline 1 and 2 determined the effectiveness of the cleaning protocol used. Sampling point's ***b*** (prior to cleaning) and ***c*** (after cleaning) determined the effectiveness of cleaning after Session 1. Sampling points ***d*** (prior to cleaning) and sampling ***a*** of the following day (after cleaning) determined the effectiveness of cleaning after Session 2. Tables 48 (EPI), 49 (MTX) and 50 (CP) on the following pages show the amount contamination recovered before, and after, each cleaning procedure was applied.

Table 48. Amount of EPI Contamination Recovered from the Isolator before and after Session Cleaning (Baseline 1 and 2)

Area	End of Session 1 Clean								End of Session 2 Clean							
	Baseline 1				Baseline 2				Baseline 1				Baseline 2			
Isolator base, ng cm^{-2}																
b	0.59	<0.002	0.07	0.04	ND	<0.002	ND	ND	0.11	0.08	<0.002	0.06	ND	ND	ND	ND
a	0.17	0.09	0.05	0.07	ND	<0.002	ND	ND	0.20	0.08	0.05	ND	ND	<0.002	ND	ND
Door, outer right, ng cm^{-2}																
b	0.07	<0.003	ND	ND	ND	ND	ND	ND	0.05	ND	ND	ND	ND	ND	<0.003	ND
a	0.07	ND	ND	0.07	ND	<0.003	ND	ND	0.06	ND	ND	ND	ND	ND	ND	ND
Door, outer left, ng cm^{-2}																
b	0.01	ND	<0.003	ND	ND	ND	ND	ND	0.05	ND	ND	ND	ND	ND	ND	ND
a	<0.003	<0.003	ND	0.07	ND	<0.003	ND	ND	<0.003	ND	ND	ND	<0.003	ND	ND	ND
Door, inner right, ng cm^{-2}																
b	0.08	0.06	ND	ND	ND	<0.003	ND	ND	0.07	ND	ND	ND	ND	ND	0.05	ND
a	<0.003	ND	ND	0.07	ND	ND	ND	ND	<0.003	<0.003	ND	ND	<0.003	ND	ND	ND
Door, inner left, ng cm^{-2}																
b	0.08	0.07	<0.003	<0.003	ND	<0.003	ND	ND	0.07	ND	ND	ND	ND	ND	ND	<0.003
a	ND	ND	<0.003	<0.003	ND	<0.003	<0.003	ND	0.05	ND	ND	ND	<0.003	ND	ND	ND
Sleeve, right, ng per sleeve																
b	711	41.0	42.5	ND	ND	ND	ND	ND	90.5	ND	ND	ND	ND	<2.0	<2.0	ND
a	89.5	41.5	45.0	49.0	ND	<2.0	ND	ND	106	41.5	ND	ND	ND	ND	ND	ND
Sleeve, left, ng per sleeve																
b	398	<2.0	<2.0	<2.0	ND	ND	ND	<2.0	81.0	ND	ND	53.5	ND	<2.0	ND	ND
a	86.0	<2.0	ND	49.5	ND	<2.0	ND	ND	44.0	<2.0	<2.0	ND	ND	ND	ND	ND

b = before cleaning
a = after cleaning
ND = none detected

EPI LoD (ng cm^{-2}) = 0.001 (base, hatch door), 1.0 ng per sleeve

Table 49. Amount of MTX Contamination Recovered from the Isolator before and after Session Cleaning (Baseline 1 and 2)

	End of Session 1 Clean								End of Session 2 Clean							
Area	**Baseline 1**				**Baseline 2**				**Baseline 1**				**Baseline 2**			
Isolator base, ng cm^{-2}																
b.	ND	ND	0.26	<0.04	ND	ND	ND	ND	<0.04	ND	0.19	0.09	ND	ND	ND	0.24
a	ND	ND	0.18	<0.04	ND	ND	ND	0.27	ND	0.33	0.13	ND	ND	0.60	0.24	ND
Door, outer right, ng cm^{-2}																
b	ND	ND	ND	0.07	<0.07	ND	<0.07	<0.07	<0.07	ND	ND	ND	ND	ND	ND	0.15
a	ND	ND	ND	<0.07	<0.07	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.11	ND
Door, outer left, ng cm^{-2}																
b	ND	ND	ND	<0.07	<0.07	ND	<0.07	0.16	ND	ND	ND	ND	ND	ND	ND	0.24
a	ND	ND	ND	<0.07	ND	ND	ND	0.19	ND	ND	ND	ND	ND	ND	0.14	ND
Door, inner right, ng cm^{-2}																
b	ND	ND	ND	0.10	ND	ND	ND	0.19	ND	ND	ND	0.22	ND	8.04	ND	0.08
a	<0.07	ND	ND	ND	ND	ND	ND	<0.07	ND	ND	ND	ND	ND	ND	0.20	ND
Door, inner left, ng cm^{-2}																
b	ND	ND	ND	<0.07	ND	ND	<0.07	<0.07	ND	ND	ND	ND	ND	ND	ND	<0.07
a	ND	ND	ND	<0.07	ND	ND	ND	<0.07	ND	ND	<0.07	ND	ND	ND	0.15	ND
Sleeve, right, ng per sleeve																
b	ND	ND	ND	<50.0	ND	ND	<50.0	ND	ND	ND	ND	ND	ND	ND	ND	ND
a	ND	ND	<50.0	ND	ND	ND	102	<50.0	ND	ND	ND	ND	ND	ND	198	ND
Sleeve, left, ng per sleeve																
b	ND	ND	<50.0	<50.0	<50.0	ND	<50.0	ND	<50.0	ND	ND	ND	ND	ND	ND	716
a	ND	ND	ND	282	ND	ND	158	211	ND	ND	<50.0	ND	ND	ND	<50.0	ND

b = before cleaning
a = after cleaning
ND = none detected

MTX LoD (ng cm^{-2}) = 0.02 (base), 0.04 (hatch door), 25.0 ng per sleeve

Table 50. Amount of CP Contamination Recovered from the Isolator before and after Session Cleaning (Baseline 1 and 2)

Area	End of Session 1 Clean								End of Session 2 Clean							
	Baseline 1				Baseline 2				Baseline 1				Baseline 2			
Isolator base, ng cm^{-2}																
b.	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, outer right, ng cm^{-2}																
b	ND	ND	ND	ND	ND	ND	269	ND	ND	ND	ND	ND	ND	ND	ND	ND
a	ND	ND	117	ND	426	ND	174	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, outer left, ng cm^{-2}																
b	ND	ND	ND	ND	ND	ND	190	ND	ND	ND	135	ND	ND	ND	393	ND
a	ND	ND	ND	ND	ND	ND	215	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, inner right, ng cm^{-2}																
b	ND	ND	ND	ND	ND	ND	302	ND	ND	ND	ND	ND	ND	ND	982	ND
a	ND	ND	ND	ND	ND	ND	368	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, inner left, ng cm^{-2}																
b	ND	ND	ND	ND	ND	ND	237	ND	ND	ND	ND	ND	ND	ND	2,034	ND
a	ND	ND	ND	ND	ND	ND	221	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve, right, µg per sleeve																
b	ND	ND	ND	ND	ND	ND	304	ND	ND	ND	ND	ND	ND	ND	ND	ND
a	ND	ND	ND	ND	ND	ND	264	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve, left, µg per sleeve																
b	ND	ND	ND	ND	ND	ND	278	ND	ND	ND	ND	ND	ND	ND	ND	ND
a	ND	ND	ND	ND	134	ND	266	ND	ND	ND	ND	ND	ND	ND	ND	ND

b = before cleaning
a = after cleaning
ND = none detected

CP LoD (ng cm^{-2}) = 9.5 (base), 17.5 (hatch door), 12.5 µg per sleeve

The effectiveness of the cleaning protocol applied in Baseline periods of the study was assessed by comparing surface contamination in the isolator (ng per square centimetre, or ng or µg per sleeve) before and after cleaning during Baseline 1 and 2. The frequency of cases when contamination was removed was determined using the Chi Squared Test. The significance level of the test was $p \leq 0.05$, which meant there was sufficient evidence to reject the null hypothesis. The hypothesis were:

H_o – cleaning does <u>not</u> remove cytotoxic contamination in the isolator

H_1 – cleaning does remove cytotoxic contamination in the isolator

The cleaning procedure applied after Session 1 did not significantly reduce contamination of EPI ($p = 0.853$), MTX ($p = 0.655$) or CP ($p = 0.317$) from areas in the isolator.

The cleaning procedure applied after Session 2 did not reduce contamination of EPI ($p = 0.371$) or MTX ($p = 0.827$) from areas in the isolator. There were insufficient valid occurrences of an ineffective cleaning procedure for CP to enable statistical assessment, as CP was removed completely (at least to below the LoD of the analytical method) in all four cases.

There was only one occasion when contamination was recovered at the end of the week to assess the cleaning procedure applied to clean the floor. On this one occasion, the cleaning procedure was effective in removing MTX contamination.

5.5.7 Acceptance of the Closed-System (PhaSeal®) Device - Questionnaire

The questionnaire (see Appendix 6) was completed by both technicians and consisted of open and closed questions. Feedback on parts 1, 2 and 3 is expressed in Tables 51, 52 and 53, respectively, below and on the following pages.

Table 51. Results of Part 1 of the Questionnaire to Determine Operators Opinion on the Use of the Closed-System (PhaSeal®) Device

Part 1. Prior to the Start of the Study	
Question 1.a. How long have you been working with cytotoxic drugs?	
Answer	*T1. 3 years*
	T2. A few days
Question 1.b. How worried are you about working with cytotoxic drugs?	
Answer	*T1. Quite happy*
	T2. Slightly bothered
Question 1.c. How safe do you feel with the current methods you have been using when working with cytotoxic drugs?	
Answer	*T1. Very safe - "very competent and well trained"*
	T2. Safe

T1 = Technician One
T2 = Technician Two

Table 52. Results of Part 2 of the Questionnaire to Determine Operators Opinion on the Use of the Closed-System (PhaSeal®) Device

Part 2. Prior to the Start of the Intervention Period	
Question 2.a.	I found the training for the closed-system device adequate
Answer	*T1. Strongly agree*
	T2. Strongly agree
Question 2.b.	I felt confident using the closed-system device after the training given
Answer	*T1. Strongly agree*
	T2. Strongly agree
Question 2.c.	Do you have any reservations about using the closed-system device?
Answer	*T1. Packaging waste*
	T2. "No, we were trained well enough to allow us to fix a problem if one occurs".
Question 2.d.	How confident do you feel that the closed-system device is protecting you?
Answer	*T1. Confident*
	T2. Very confident

T1 = Technician One
T2 = Technician Two

Table 53. Results of Part 3 of the Questionnaire to Determine Operators Opinion on the Use of the Closed-System (PhaSeal®) Device

Part 3. End of the Intervention Period	
Question 3.a. How confident are you in using the closed-system device now?	
Answer	*T1. Very confident* *T2. Very confident*
Question 3.b. I was more careful when using the closed-system device than when using the traditional 'open' method	
Answer	*T1. Disagree* *T2. Disagree*
Question 3.c. Did you have any problems when using the device?	
Answer	*T1. Time to unwrap individual product* *T2. "Only once when I separated the injector from the vial with the protector on it. I realised I was pushing down on the injector at the same time I was pulling it apart. This caused them to come apart".*
Question 3.d. Did the device hinder work in any way?	
Answer	*T1. "No, made it easier to draw up, less strain on hands"* *T2. "Not at all, I looked forward to the weeks when using PhaSeal®"*
Question 3.e. After using the closed-system device which method would you prefer to continue working with?	
Answer	*T1. PhaSeal® device – "Quicker and easier to use"* *T2. PhaSeal® device – "Although it is a little time consuming unwrapping all of the individually wrapped PhaSeal® parts. I found it was actually quicker to produce the end product. I felt a lot safer using PhaSeal® especially when drawing up liquid from vials. It was a lot easier to manoeuvre the vials whilst manufacturing. There was also less strain on the thumbs when using the PhaSeal® compared to the traditional method".*

T1 = Technician One
T2 = Technician Two

5.6 Discussion

This study was designed as a short-term study simulating the compounding of cytotoxic drugs in an isolator under UK standards of practice. The intense sampling schedule enabled a large amount of data to be collected over the whole 4-week study period. The three drugs of interest were the only ones manipulated, thereby eliminating interference from any other drugs. The method (Chapter 3, Method 2)

employed to remove cytotoxic surface contamination was novel compared to wipe sampling methods applied previously. It used a wipe impregnated with 70% (v/v) IPA, in a single recovery method, to remove the multi-surface contamination of three cytotoxic drugs. It was sensitive to measure surface contamination at nanogram levels of two of the drugs of interest. Prior to its application, it was validated for recovery from each surface (Section 3.8). All of the samples collected were assayed for all three drugs.

The data generated from the sampling schedule enabled the quantification of contamination of surfaces inside the isolator, the finished product leaving the isolator and the immediate surfaces outside the isolator. It enabled conclusions to be drawn about the effectiveness of the closed-system (PhaSeal®) device and of the cleaning protocol used.

The presence of cytotoxic contamination, particularly during the Baseline periods, on the gloves and sleeves, the base of the isolator, the hatch doors and the batches prepared, demonstrates that contamination does occur from the compounding of cytotoxic drugs in an isolator workstation. However, this study showed that the levels of contamination vary, are inconsistent, and are not predictable. There was a clear difference in the amount of contamination and the frequency of positive samples between the three drugs recovered from the surfaces inside the isolator. The frequency of EPI contamination, in terms of the number of positive samples recovered from the isolator during Baseline was the highest, followed by MTX, then CP (Figure 44, page 212). This reflects the different LoD and LoQ of the analytical method for each drug (Section 3.8.4); the method applied to quantify EPI was the most sensitive, followed by MTX then CP. A review of environmental studies

reported the positive number of wipe samples taken from hospital pharmacies to range between 0 - 100%.[8] Other studies have reported a range of positive samples for CP from surfaces *i.e.* 29%, 21%, 95% and 94%;[124-126] and 2%, 56%, and 60% for MTX.[108;109;189] These samples were taken from established hospital pharmacies where baseline levels of contamination may possibly be higher and there were longer intervals between sampling points *i.e.* the end of the working day over a year,[20] twice weekly,[62] every 4 weeks,[78] at various monthly intervals.[124] The Baseline levels in the isolator and clean-room in this present study were zero prior to the start of the study *i.e.* completely free from cytotoxic contamination and ideal for research purposes. The frequency of monitoring was very intense over a short period of time *i.e.* four times daily over a period of 4 weeks (Section 5.4.7 and 5.4.8), not allowing much time for the potential build-up of contamination between monitoring.

The levels of contamination in this study were sufficient to make comparisons between the two different methods for the reconstitution and dilution of the three drugs. For each drug, a significant reduction in the number of samples, which tested positive during Baseline compared to Intervention, was observed (Figure 44, page 212). Although the frequency of the number of positive samples of CP was the lowest of the three drugs, the total amount of CP recovered during Baseline 1 was the highest for the study, indicating that CP was the most contaminating drug. This was supported by the fact that CP contributed more cumulative contamination to isolator surfaces per mg prepared over the whole week (67.9 ng), than EPI (44.1 ng) or MTX (22.3 ng) during Baseline 1. This may reflect the way in which CP was prepared *i.e.* it is a lyophilised powder, and requires an extra manipulation *i.e.* reconstitution prior to dilution, compared to EPI and MTX which were presented in solution form and simply diluted. The lower frequency of

the number of positive samples may be due to the lack of sensitivity of the CP assay method; contamination may have been present but it was not measurable. On occasions when CP was recovered, the amount measured was well above the LoQ of the analytical method *i.e.* there were no samples that were positive for CP, but could not be quantified. If a more sensitive method had been employed or if higher recoveries had been achieved to give a lower LoD, it is likely that a higher frequency of positive samples would have been recorded.

Interestingly, no CP contamination was recovered from the isolator base, despite this being a positive source of contamination of EPI and MTX. In the isolator, most CP contamination was recovered from the hatch doors (both sides) - Table 35 (page 214), and the 'tray in' and 'tray out' - Table 45 (page 230). Worryingly, the highest amounts of CP contamination were recovered from the support gloves used outside the isolator. Levels on these gloves tested positive during both the Baseline and Intervention weeks of the study, indicating a pre-contaminated source of CP prior to any manipulations being carried out in the isolator. It is known that high external contamination on drug vials as received from the manufacturer, are a source of pre-contamination, and have been previously reported for CP.[41;43;45] In Section 2.4.3.7 of this thesis, contamination was reported on vials, when measured in 2 out of 7 cases in hospital pharmacies in the UK. Cytotoxic drug contamination has also been found on packaging[40;63] and contaminated vials have been suggested to be an influencing factor on data from other surface contamination studies.[26;126] The likelihood of external contamination on the vial may explain contamination on the 'tray in' where high quantities of CP were recovered on several occasions.

The high quantities of CP found on the support gloves is of great concern as this is a potential source of dermal exposure. Typically, the same pair of gloves

would be worn for the whole session, unless glove integrity was visibly compromised, and the gloves would be changed. However, in this study, double gloving was practiced when working in the isolator. The outer gloves used in the isolator were made of latex, a thick glove especially designed for handling cytotoxics, and underneath a thinner nitrile glove was worn (contamination was only measured for on the thicker, latex isolator, gloves). However, even these thick latex gloves would have a finite breakthrough time. Certainly, the practice of double gloving and the higher temperatures achieved from the running of the isolator would cause the hands to sweat and this may influence the permeation of gloves.[38] The support gloves were made of nitrile but they were considerably thinner and possibly offered less protection. The high levels of CP contamination found on these support gloves used outside the isolator would pose the risk of dermal exposure. A session may have lasted a number of hours and OSHA recommends that gloves are changed every hour.[79] Studies have shown breakthrough times through latex gloves for CP and MTX as rapid as 10 min and 62 min.[30] Unlike the isolator gloves, the support gloves were worn in the clean-room, a larger area in which to transfer contamination. Contaminated support gloves would potentially transfer contamination onto any areas touched and cause a secondary contamination event *i.e.* areas in the clean-room or, on items sprayed in for subsequent batch preparation in the same session.

Significant levels of CP were recovered from the 'tray in' during Day 3 of Baseline 2. The positive testing of the 'tray in' for CP contamination and corresponding contamination recovered in the isolator during the same sessions, may also be a consequence of external contamination on vials.

The liberal spraying of IMS on vials prior to transfer into the isolator may also be a method of transferring contamination. The wetting with IMS on heavily

contaminated vials may act as a medium to carry any contamination into the tray or onto the base when transferred into the isolator. Significant amounts of CP were also recovered during sessions when CP was not manipulated. Airborne levels of CP have periodically been reported when CP was not the drug being manipulated.[34] It has been suggested that CP may vaporise as it has a low vapour pressure,[34] and diffuse through the HEPA filter.

Anomalous results have been previously reported before when large quantities of CP were found on gloves used for handling vials which did not contain CP. It was suggested that the cross-contamination of vials may occur during the manufacturing process. This may be prevented or reduced by washing the vials after the manufacturing process and would also reduce the levels of pre-contamination provided by the corresponding drug.[45] The importance of CP in this study was as a marker drug for comparison with other studies. Although it was not detected frequently, a reduction in contamination when using the closed-system (PhaSeal®) device was observed, as with other studies.[20;67;78;124]

The first Baseline week, using the open-system, showed high levels of EPI contamination (Table 35, page 214). The largest amount of EPI contamination was recovered on Day 1, and the amount and frequency of contamination decreased throughout the week. The amount of EPI recovered from the base, right sleeve and both gloves, and also the frequency of contamination, was lower during Baseline 2. It may be suggested that the technicians, after using the closed-system (PhaSeal®) device for one week, were more careful when using the open-system. However, this suggestion cannot be supported by the fact that the amount of MTX and CP

contamination recovered was higher during the second Baseline week. Certainly, on Day 3 of Baseline 2, a large amount of CP was recovered.

The median surface levels recovered of EPI and MTX contamination were highest on the gloves and sleeves, and on the inner right hatch door (Table 35, page 214). During Baseline 1, EPI was recovered consistently from the isolator gloves, which would explain the frequent contamination recovered from the syringes. On several occasions, especially during Baseline 2, the levels of EPI were below the LoD of the analytical method and traces, which were not measurable, were found frequently on the hatch doors where less contamination would be expected. No MTX was recovered from the support gloves during any week of the study (Table 47, page 232). The size and the number of MTX vials was smaller than the size and number of EPI and CP vials required for the study, resulting in a smaller potentially pre-contaminated surface area. EPI, MTX and CP were all supplied by different manufacturers, and variations in washing could explain differences in external contamination on vials of different drugs and between manufactures.[41] None of the vials of EPI, MTX or CP were supplied with a coating shrunk around the vial to contain any contamination, and there is no standardised cleaning procedure between manufacturers. It is possible that the MTX vials received may not have been as contaminated as the CP vials because of the filling process. The process of filling vials with lyophilised powder (*i.e.* CP) may cause dusting, which may be more contaminating than filling a vial with a liquid *i.e.* EPI or MTX. It was not anticipated that such high levels of CP would be recovered from areas outside of the isolator *i.e.* support gloves in this study. Including vials as part of the wipe sampling schedule would have confirmed if the vials were the source of pre-contamination. Cleaning of

the vials prior to the start of the study may have removed this potential pre-contaminating source. These options should be considered in future studies.

Isolators are designed for containment, and any contamination generated or taken into the isolator should pose no threat to operators. However, isolators do leak to some extent[110] and this may partly explain the presence of contamination on the floor. Floor contamination recovered when cytotoxic drugs were compounded in a negative-pressure isolator (also recovered when a positive-pressure isolator was used) was found by another study. The levels of CP and MTX recovered were 117 ng m^{-2} (median) ranging from 22.0 ng m^{-2} to 290 ng m^{-2}, and 58 ng m^{-2} (median) ranging from 40.0 ng m^{-2} to 674 ng m^{-2} respectively.[26] These levels are considerably higher than the levels of CP and MTX recovered from the floor in this present study (Table 47, page 232).

It is evident that the external contamination of syringes occurs during preparation and this is highly variable. Syringes making up the batch were sometimes contaminated infrequently *e.g.* 1 syringe from a batch of MTX was found to be contaminated with 259 ng of the corresponding drug. In other cases contamination was frequent and consistent *e.g.* all the syringes from EPI batch 4 during Baseline 1 were contaminated (range = 42.0 to 78.5 ng). The bagging of each batch of syringes would retain the contamination until the bag was re-opened, but any highly contaminated syringes may potentially contaminate other, non-contaminated syringes, in the batch, unless packaged as individual units. The external cross-contamination of batches was also found to occur, although less frequently than the contamination of syringes from the corresponding drug. Cross-contamination is likely to occur from handling with contaminated gloves or placing the syringes onto contaminated surfaces. EPI was the most frequent contaminant and cross-

contaminant recovered from batches i.e. 9 of the 10 syringes of CP (batch 14) were cross-contaminated with similar amounts of EPI during Baseline 1, day 3. This cross-contamination is difficult to explain as the CP batch was made prior to any EPI preparation that day. In fact, the last batch of EPI was prepared in the morning session of the previous day, and since then the isolator had been cleaned twice and the gloves changed.

During the Intervention weeks, there was no positive presence of CP on any of the syringes (Table 43, page 224). EPI was positive on 3 syringes but could not be quantified as it was below the LoQ of the analytical method. During Intervention 2, 81.5 ng of MTX was recovered from one syringe, on the last batch prepared that week. There was no other MTX contamination recovered from the surfaces in the isolator during the whole week of Intervention 2, except for a trace on the right sleeve at the beginning of the week, therefore implying the isolator was free from MTX contamination. This anomalous result could potentially be explained by a contaminated vial. However, no contamination was recovered from the support or isolator gloves to support this. The syringe may have been placed on a surface of the isolator, which had not been sampled from, as wipe samples were taken only from 21% of the isolator base. In previous studies with the PhaSeal® device, the occasional positive sample has been reported.[125] In this present study, the occasional positive sample during Intervention may be carryover from Baseline due to the ineffectiveness of the cleaning procedure.

The closed-system (PhaSeal®) device, used in conjunction with an isolator was able to contain and control the environment inside the isolator during drug preparation and reduce contamination on the finished product leaving the isolator. Although control could only be exerted in the isolator, the reduced contamination

found on batches would also reduce exposure to personnel involved in the transportation, handling and administration of the finished product.

The results suggest that the cleaning protocol applied in this study was not fully effective in removing surface contamination of all three drugs. The two cleaning agents (Klercide CR-B and Klerclean neutral detergent) and IMS are used commonly for cleaning after the manipulation of cytotoxic drugs in the facilities which responded to the survey (Chapter 2). Wipe studies carried out with these two cleaning agents in Chapter 4, demonstrated that these two agents and IMS have the potential to be effective in removing cytotoxic contamination, where EPI and CP were two of the contaminating drugs investigated (Table 27, page 166). However, these studies were applied to a small surface area and the contamination was visible to the naked eye. The task of cleaning an isolator is very different. An isolator is awkward to clean manually, using the sleeve/glove port access makes it a clumsy task, some areas are inaccessible, and it is difficult to be methodical and apply the same amount of pressure over the whole surface. It may be possible that some areas were not reached or that the procedure applied was simply spreading the contamination around. The sampling procedure did not cover all of the surface area of the isolator therefore, cleaning would overlap swabbed and un-swabbed areas and if contamination was being spread, it is possible that it could have been transferred onto the swabbed areas. This would explain the findings of drug contamination when the corresponding drug had not been prepared in the preceding session. In most cases, cytotoxic contamination was not visible to the naked eye and decontamination was dependent on the cleaning procedure.

It may also be possible that the contamination was persistent and that the cleaning agents applied in this study were not appropriate to collect or degrade these

cytotoxic molecules. Studies on these two cleaning agents in Chapter 4 showed that they were not effective in degrading cytotoxic drugs, once more EPI and CP were two of the drugs investigated (Section 4.5.3). It must be noted that taking swabs during sampling points ***c*** and ***a*** would have removed some of the contamination prior to cleaning. As a result, the number of positive occasions when the cleaning procedure was effective may be an over-estimation, as the removal of contamination may have been due to the removal by the sampling procedure and not by cleaning.

5.6.1 Levels of Cytotoxic Contamination Recovered – A summary

Table 54 on the following page summarises the range of contamination recovered from the isolator, areas outside the isolator and syringe batches during the combined Baseline 1 and 2, and Intervention 1 and 2 periods.

The range of MTX contamination recovered in this study is in agreement with levels which were recovered from a positive-pressure isolator *i.e.* ND to 8.61 ng cm^{-2} under French standards of practice.[59] This supports the conclusion that there is no distinction in terms of risk to the operator between the differential operating pressure of the isolator in relation to the pharmacy atmosphere.[26] Although, contrary to this, the levels of CP are significantly higher *i.e.* ND to 2,034 µg cm^{-2} in this present study, compared to the range of CP contamination recovered from a positive-pressure isolator *i.e.* 0.16 to 6.55 ng cm^{-2}.[59]

Table 54. Summary of the Range of Contamination Recovered During the Baseline and Intervention Periods

Area		Baseline	Intervention
Isolator	EPI	ND - 0.59 ng cm^{-2}	ND - 0.11 ng cm^{-2}
		ND - 1,932 ng per sleeve/glove	ND - 171 ng per sleeve/glove
	MTX	ND - 8.04 ng cm^{-2}	ND - 0.07 ng cm^{-2}
		ND - 1,559 ng per sleeve/glove	ND - <50.0 ng per sleeve/glove
	CP	ND - 2,034 ng cm^{-2}	ND
		ND - 304 µg per sleeve/glove	ND - 192 µg per sleeve/glove
Outside	EPI	ND - 0.19 ng cm^{-2}	ND
Isolator		ND -13,268 ng per glove	ND - 1,046 ng per glove
	MTX	ND - 11.3 ng cm^{-2}	ND - 0.39 ng cm^{-2}
		ND	ND
	CP	ND - 384 ng cm^{-2}	ND - 214 ng cm^{-2}
		ND - 1,356 µg per glove	46.0 - 1,476 µg per glove
Batches	EPI	ND - 1,807 ng per syringe	0 - <2.0 ng per syringe
	MTX	ND - 259 ng per syringe	ND - 81.5 ng per syringe
	CP	ND - 75.0 µg per syringe	ND

ND = none detected

5.6.2 Effectiveness of the Closed-System (PhaSeal®) Device – A summary

Statistical tests comparing the Baseline and Intervention weeks of the study, demonstrated the closed-system (PhaSeal®) device to be significantly effective in preventing the release of contamination from the compounding of all three drugs in

the isolator (Section 5.5.2). A summary of the outcome of the effectiveness of the closed-system (PhaSeal®) device is shown in Table 55 below. Where there were a sufficient number of statistical cases the p value was quoted (p values equal to or below <0.05 were considered to be significant). Where there were not enough statistical cases the reduction in the number of positive cases is shown.

Table 55. Effectiveness of the Closed-System (PhaSeal®) Device in Preventing Cytotoxic Contamination

		Statistical Outcome		
Area		**EPI**	**MTX**	**CP**
Isolator	B1 vs I1	p = 0.000	p = 0.000	[a]
	B2 vs I2	p = 0.003	p = 0.000	p = 0.002
	B1+2 vs I1+2	ND	ND	p = 0.001
Batches of Corresponding Drug		p = 0.000	5 → 1 [a]	2 → 0 [a]
Batch Cross-Contamination		11→0 [a] (MTX) p = 0.012 (CP)	6 → 0 [a] (EPI) 1 → 0 [a] (CP)	4→0 [a] (EPI) ND [b] (MTX)
Cleaning				
	Session 1	p = 0.853	p = 0.655	p = 0.317
	Session 2	p = 0.371	p = 0.827	4 → 0 [a]
	Floor	ND [b]	1→ 0 [a]	ND [b]

[a] = Insufficient number of positive cases above the LoD to carry out statistical test
[b] = No positive cases
B1 vs I1 = Baseline week 1 versus Intervention week 1
B2 vs I2 = Baseline week 2 versus Intervention week 2
B vs I = Baseline (week 1+2) versus Intervention (week 1+2)
ND = not determined

There were incidences when statistical analysis could not be carried out *i.e.* due to the lack of positive samples. It may be possible that the contamination was not present, or that it was below the LoD of the analytical method. Nethertheless, there

was a significant reduction in the levels of contamination recovered from the isolator between the two systems for the reconstitution and preparation of cytotoxic drug containing syringes and infusion bags. This was true for two drugs (EPI and MTX), novel to usage with the closed-system (PhaSeal®) device, and was supported by the results for CP found by other investigators.[20;62;67;78;124;125] It should be noted that the closed-system device would not be effective in reducing contamination arising from the exterior wall of drug vials.

5.6.3 Acceptance of the Closed-System (PhaSeal®) Device

Feedback from the technicians in this study supported acceptance of the closed-system (PhaSeal®) device and preference of its usage to the 'open' technique – see Tables 52 and 53 (pages 239 and 240). This was true for an experienced technician and a technician new to the role. Technician One had 3 years of experience of working with cytotoxic drugs. She was quite happy working with cytotoxic drugs and felt very safe using the open-system, which she has always used in previous employment. She considered herself very competent and well trained in her technique. Technician Two was a pharmacist who had less experience of working with cytotoxic drugs. She was slightly bothered about working with these drugs but felt safe with the open-system and the safety protection provided. However, after seeing how the closed-system (PhaSeal®) device worked, she was no longer keen on using the open-system.

Prior to the start of the Intervention period, both technicians found the training given before use of the device adequate, and afterwards felt confident in using the closed-system (PhaSeal®) device. Technician One felt confident that the device was protecting her from contamination, but found the packaging problematic when opening it to remove the device. Technician Two felt very confident that the

device was protecting her from contamination, she did not have any reservations about the device as the training given also covered troubleshooting and felt that if a problem did occur it would be 'fixable'.

After two weeks of usage with the closed-system (PhaSeal®) device, both technicians felt very confident with using it and preferred this method to the open-system. Again, the only drawback was the individual packaging which was problematic when many vials were used requiring the removal of many protectors from packaging. Opening of the packaging in the isolator was hindered by the wetting of the packaging caused by IMS saturation when spraying in. This problem was referred to the manufacturer.

Both technicians found it quicker to produce the end-product when using the device (apart from the unwrapping of the components) and found it easier to draw up liquid, relieving strain on the hands. Technician Two actually looked forward to the Intervention weeks when using the device. She felt safer using the device, especially when drawing up liquid from the vials, and it made the vials much easier to manoeuvre during compounding.

5.6.4 Potential Surface Contamination Risk Assessment for Pharmacy Technicians - A Theoretical Projection of Data

When data have been lacking because of the time it would take to collect, short-term available data have been extrapolated to calculate a long-term risk.[22] The modelling of data in such a way enabled a cancer risk from exposure to CP to be determined.[22] This information otherwise would only have been generated from observation over a long period of time.

The total amount of drug compounded (Table 31, page 197) and the amount of surface contamination generated by EPI, MTX and CP (Appendices 7, 8 and 9,

respectively) over the two Baseline weeks using the open-system was aggregated. The data was projected to estimate a short-term risk *i.e.* over 1 year (the data from 2 weeks was multiplied by 24, assuming a working year of 48 weeks) and a long-term risk *i.e.* over 40 years (the data from 1 year was multiplied by 40, assuming a working life of 40 years). The calculation was performed for EPI, MTX and CP, grouping contamination recovered from the different surfaces into three areas of risk:

area *a* contamination recovered from surfaces inside the isolator only;

area *b* contamination recovered from all surfaces outside the isolator;

area *c* contamination and cross-contamination recovered from the external surfaces of batches of infusions.

The same calculation was performed comparatively alongside Intervention data to calculate an estimated potential reduced risk over the same time points. The results of these calculations are shown in Table 56 below, and Tables 57 and 58 on the following page.

Table 56. Amount of EPI, MTX and CP Compounded over 2 Weeks, and Estimated over 1 Year and 40 Years

	Amount of Drug Compounded		
Drug	**2 Weeks**	**1 Year**	**40 Years**
EPI	0.74 g	17.8 g	710 g
MTX	0.99 g	23.6 g	947 g
CP	5.3 g	127 g	5,088 g

Table 57. Amount of Drug Contamination Produced during Baseline over 2 Weeks, and Amount Estimated over 1 Year and 40 Years

Drug	Amount of Drug Recovered from Surfaces in Area		
	2 weeks	**1 year**	**40 years**
EPI			
area *a*	21,750 ng	0.52 mg	20.9 mg
area *b*	20,006 ng	0.48 mg	19.2 mg
area *c*	4,657 ng	0.11 mg	4.47 mg
MTX			
area *a*	23,252 ng	0.56 mg	22.3 mg
area *b*	6,057 ng	0.15 mg	5.81 mg
area *c*	537 ng	0.01 mg	0.52 mg
CP			
area *a*	1,170 µg	28.1 mg	1,123 mg
area *b*	25,087 µg	602 mg	24,084 mg
area *c*	343 µg	8.23 mg	329 mg

Table 58. Amount of Drug Contamination Produced during Intervention over 2 Weeks, and Amount Estimated over 1 Year and 40 Years

Drug	Amount of Drug Recovered from Surfaces		
	2 weeks	**1 year**	**40 years**
EPI			
area *a*	510 ng	0.01 mg	0.49 mg
area *b*	1,411 ng	0.03 mg	1.35 mg
area *c*	ND	ND	ND
MTX			
area *a*	1,499 ng	0.04 mg	1.44 mg
area *b*	611 ng	0.15 mg	0.59 mg
area *c*	81.5 ng	0.002 mg	0.08 mg
CP			
area *a*	ND	ND	ND
area *b*	23,768 µg	570 mg	22,817 mg
area *c*	ND	ND	ND

ND = none detected

Implementation of the closed-system (PhaSeal®) device would reduce the risk of surface contamination (>LoD) in area *a* of EPI and MTX contamination by 97.7% and 93.6%, respectively, and would eliminate CP contamination.

Although the closed-system (PhaSeal®) device would not exert any direct control over the environment outside of its immediate use, EPI contamination was reduced by 92.9%, MTX contamination by 90.0%, and CP contamination by 5.3% in area *b*.

Implementation of the closed-system (PhaSeal®) device would eliminate external surface contamination (>LoD) on batches of syringe infusions of EPI and CP, and reduce MTX contamination by 84.8%.

The approach of short and long-term projection of the data from this study would be of importance to those who would consider an occupation of working with cytotoxic drugs either short-term or long-term. It was recognised that this can only be an estimate, and there were some drawbacks in this estimation due to the following assumptions that were made:

i. batches of the same size and of the same quantities of these three drugs were prepared week in, week out; firstly over 1 year, then over a 40 year period;
ii. no effect of other drugs used in the unit over this time was considered;
iii. the technician worked everyday for 40 years, with only 4 weeks of holidays per year;
iii. there was no change in the compounding method, use of personal protective equipment, cleaning procedure or facilities where the cytotoxic drugs were compounded;

iv. the contamination was consistently produced, and there were no influences such as reported spillages.

In this study, the areas swabbed, represented 21% of the isolator base, a small area of the floor, the cuffs of the sleeves, and one third of the batches leaving the isolator. Therefore, this contamination risk is likely to be an under-estimation of the actual risk in practice. Furthermore, it may be possible that the results from this study are a slight under-indication of those that may be recovered from an established hospital environment after long-term continued use of the facilities for the compounding of cytotoxic drugs. However, for comparative purposes, the projection is useful in estimating the effect of new technology in the form of the closed-system (PhaSeal®) device. It demonstrates how the implementation of measures such as a closed-system (PhaSeal®) device in comparison with current practice may reduce or eliminate the potential risk in three main areas. The Baseline data calculates a potential risk if no successful changes were made and was necessary to highlight the potential risk if current practice does not improve. Of course, there will be the influence of changes in practice, which might include among others, improved personal protective equipment, less use of cytotoxics and the increased use of oral drugs and biological agents.

5.7 Conclusion

Contamination was recovered from all surfaces inside the isolator after the compounding of cytotoxic drugs when using the open-system. The first contaminating event of a cytotoxic contamination-free isolator occurred rapidly *i.e.* after the first session on the first day of batch production. The cytotoxic contamination recovered from the isolator base, sleeves, hatch doors and gloves was

found to be inconsistent, and was not as the result of any spillages or visible leakages. Once present, it was resistant to removal by the cleaning protocol employed.

The contamination of the external surface of the finished product also occurs in an isolator. Contamination may not only arise from the corresponding drug prepared in the batch, but from the cross-contamination of other drugs prepared in the previous sessions or in the same session.

High levels of surface contamination recovered from areas outside the isolator indicated a pre-contaminated source. The suggested source was the supply of drug vials with external surface contamination. Use of a closed-system fluid-transfer device would not reduce contamination from this source.

The implemented closed-system (PhaSeal®) device exerted control in its immediate environment *i.e.* in an isolator, significantly reducing the levels of contamination on all surfaces. It also eliminated contamination on the external surface of batches of two of the three drugs analysed, and reduced the frequency of surface cross-contamination on batches leaving the isolator. This was true for all of the test drugs, including two drugs (EPI and MTX), which were novel to usage with the closed-system (PhaSeal®) device, and also for CP where results were in accordance with previous studies.[20;62;67;124;125] Operators of the closed-system (PhaSeal®) device preferred it to the open-system.

Isolators have the capacity of protecting immediate external areas from multi-cytotoxic drug surface contamination. When used in conjunction with a closed-system (PhaSeal®) device, the frequency and magnitude of cytotoxic drug contamination inside the isolator and the surface of prepared infusions leaving the isolator were diminished.

6. Effectiveness of a Closed-System (PhaSeal®) Device in Reducing Product Cross-Contamination of Batches in an Isolator

6.1 Introduction

There has been speculation about the possible product cross-contamination that may occur in an isolator.[190] Regulatory authorities advise against sharing an isolator where cross-contamination may present a risk, and expect to see the use of separate dedicated facilities.[110;135] However, it is not mandatory for an already financially stretched NHS to dedicate one isolator/BSC for the preparation of potentially infectious or biological agents, and another for chemotherapeutic agents in all pharmacies. Increasingly, a number of clinical trials with agents of biological origin (protein, viral drug-delivery vectors) are ongoing in the UK. Consequently, cytotoxics may share isolators with other drugs *i.e.* MABs and gene therapy agents, presenting the potential risk of product cross-contamination occurring. The occurrence of drug solution cross-contamination has already been suggested. It was implied that bacille Calmette-Guérin (BCG) contaminated MTX, after the sequential preparation of both in a BSC on the same day. The inadvertent inoculation of BCG during the administration of intrathecal MTX resulted in two children developing BCG-associated meningitis.[191]

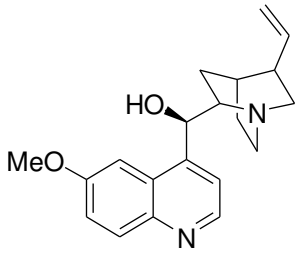
Results reported in this thesis have shown that the hospital pharmacies, which responded to the questionnaire, are already preparing MABs, occasionally in the same isolator as cytotoxic drugs, if separate facilities are not available (see Section 2.4.3.4). It has also been demonstrated that cross-contamination on the external surfaces of batches prepared in the same isolator can occur. Implementation with the closed-system (PhaSeal®) device was successful in preventing the occurrence or reducing the

number of positive cases of external surface cross-contamination on pharmaceutical dosages of all of the cytotoxic drugs compounded (see Section 5.5.4).

Fluorescent indicator solutions have been used to identify the sources and routes of contamination when evaluating the techniques used to reconstitute and dilute cytotoxic drugs.[32;60] These compounds fluoresce in UV light, highlighting spillages or poor operator technique. Pre-prepared test kits containing these fluorescent dyes are useful to train, evaluate and improve operator skills.[11;192]

Fluorescence spectrophotometry is a selective detection method, which can be used to quantify strongly fluorescent compounds in the presence of a non-fluorescent medium.[104] Quinine sulfate (QS) is a strongly fluorescent molecule due to its extended chromophore and rigid structure. Its chemical structure is shown in Figure 45 below. It is a stable compound and due to its fluorescent properties can be measured at low concentrations of 1.0 to 2.0 ng mL^{-1}.[104;193] QS was selected as the contamination indicator in this study as it exhibits UV absorption and fluorescence emission spectra.[104]

Figure 45. Chemical Structure of Quinine Sulfate



This study was carried out simulating the dilution of two different drug solutions *i.e.* WFI and QS, sequentially in the same isolator. Batches were prepared

using the closed-system (PhaSeal®) device and the workload was repeated using the traditional open-system syringe/needle technique. QS was used as an indicator to identify surface contamination in the isolator, and solution cross-contamination of the finished product.

6.2 Objectives

The objectives of this study were to achieve the following:

i. to determine if there is a significant risk of product cross-contamination occurring when different products are made in the same isolator,
ii. to identify the main areas, and the frequency and point of surface contamination when preparing batches in an isolator, and
iii. to evaluate the effectiveness of a closed-system (PhaSeal®) device for fluid-transfer in reducing this risk.

6.3 Materials

Chemicals

Quinine sulfate powder (lot 439257CM) was obtained from BDH, Poole, UK.

Consumables

Macoflex® 100 mL polyolefin intravenous infusion bags, containing 0.9% sodium chloride isotonic solution (lot 06J12D) were obtained from Maco Pharma, Twickenham, UK. Viaflo® polyolefin infusion bags, containing water for injections (lot 06116E1J) were obtained from Baxter Healthcare Ltd, Berkshire, UK.

Equipment

A UV scanning lamp with settings at 254 nm and 365 nm was from P.W Allen, London. The fluorescence spectrophotometer, model 204-S (serial no. 813-07), and 150-xenon power supply (serial no. 002) were manufactured by Perkin Elmer Instruments, Beaconsfield, UK. The digital camera was a Nikon Coolpix 3200 (serial no. 4486420).

6.4 Study Design

The study (batch preparation and the scanning of surfaces) was undertaken in a controlled GMP-compliant clean-room environment tested to EU Class Grade B[135] in an academic setting at the Department of Pharmacy, Kingston University, London, UK. The study was restricted to an isolator operating under negative-pressure relative to external atmospheric pressure with external ducting to the outside environment. The environment and the setup was the same as described in Section 5.4.1. Cross-contamination of the product was measured at the Department of Pharmacy and Pharmacology, University of Bath. UK.

The manipulations investigated were the piercing of a vial and the withdrawal of solution from the vial and its transfer to an I.V infusion bag. All manipulations, by both methods *i.e.* the closed-system (PhaSeal®) method (see Section 5.1.1) and the open-system (see Section 5.1.2) were carried out on the same day by an operator with basic training in aseptic manipulations. A professional from Carmel Pharma, Gotëborg, Sweden undertook training and competency assessment with the closed-system (PhaSeal®) device. Competency was achieved prior to the start of the study, and the professional trainer was present and observant during all of the closed-system manipulations of the study.

6.5 Methods

6.5.1 Filling of Vials

Empty 100 mL vials were filled with either 55.0 mL of WFI or QS solution. The filling process was carried out in a room separate from the clean-room where the isolator was situated. The vials filled with WFI and QS were kept segregated from each other to ensure no contact throughout all stages of the study.

WFI

Firstly, the vials were filled with WFI to avoid the cross-contamination of WFI with QS. These vials were then segregated from the QS vial filling process.

Quinine Sulfate

QS powder was dissolved in WFI to give a standard concentration of 1.0 mg mL^{-1}. The vials were filled carefully with QS solution, avoiding any contact of the QS with the external surface of the vial or any immediate surfaces. The external surface of the vials and the bungs were cleaned with WFI and a dry wipe, followed by IMS 70% v/v impregnated wipes. After the vials were cleaned, the external surface of each vial was scanned for QS contamination, as described in Section 6.5.2, to confirm a clean external surface.

6.5.2 Scanning of Surfaces

A UV lamp was used to scan surfaces for QS. It had two settings at wavelengths of 254 nm or 365 nm. QS has excitation and emission wavelengths at 350 nm and 450 nm, respectively.[104] QS was observed visually to absorb more

strongly at 365 nm therefore, it was used for the scanning of all surfaces throughout the study.

All surfaces were scanned prior to the start of the study to confirm a clean environment. Glove contamination in the form of a fluorescent marker has been reported as being difficult to visualise on glove material,[32] however information regarding the type and make of glove used was not reported. To check for the visibility of QS, a latex glove of the same type to be used in the isolator for this study, was deliberated contaminated with QS. This was undertaken in a room outside the clean-room environment.

The external surface of the vials filled with QS were scanned at 365 nm immediately after cleaning. There was no visible QS contamination on the external surface of the vials, to confirm this; the vials were immersed in 250 mL of distilled water for 1 min. The immersion water was retained, and tested for fluorescent emission to verify the external surfaces were free from QS contamination.

6.5.3 Quantification of Cross-Contamination

Cross-contamination was identified as the presence of QS in infusion bags prepared with WFI. Fluorescence spectrometry was employed to quantify any QS cross-contamination. A fluorescence spectrophotometer, model 204-S with a 150 xenon power supply was used. The sensitivity was set at 0.1 and the gain was 1 p.m.

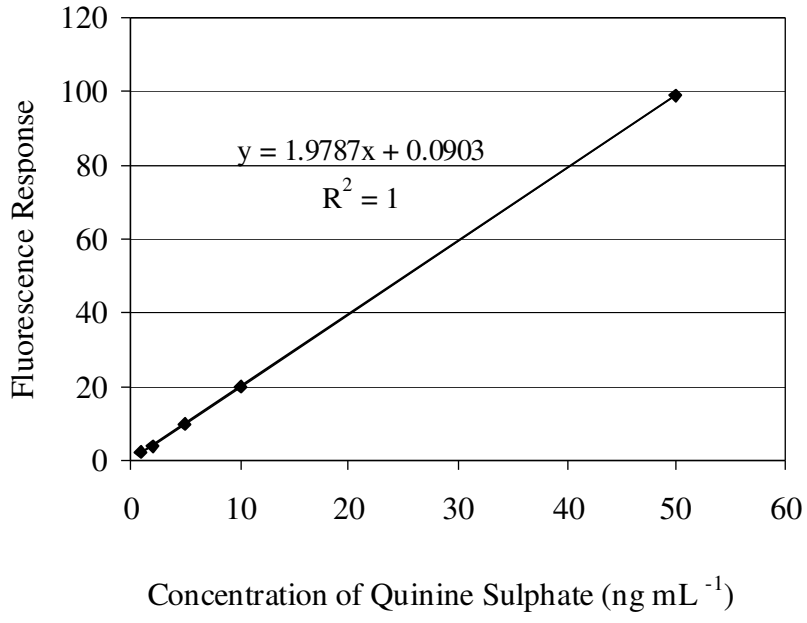
6.5.3.1 Linearity

Linearity was evaluated across the dynamic range of the spectrophotometer. The excitation wavelength of the spectrophotometer was set at 317 nm and the emission wavelength was set at 390 nm. These wavelengths were determined experimentally by measuring the excitation and emission wavelengths, which

achieved the maximum response. Standard solutions of 0.1 ng mL^{-1}, 1.0 ng mL^{-1}, 2.0 ng mL^{-1}, 5.0 ng mL^{-1}, 10 ng mL^{-1}, and 100 ng mL^{-1} QS diluted in NS were prepared. The average fluorescence response of duplicate measurements at each concentration was calculated and used to construct a calibration plot of emission intensity against the concentration of the standard solution (ng mL) of QS. Emission was measured on a scale of 0 to 110. The equation of the calibration plot was obtained by linear regression analysis of known drug concentration (*x*) versus average fluorescence response (*y*).

Fluorescence emission plotted against concentration (ng mL^{-1}) was linear over the measured concentration range of 1.0 ng mL^{-1} to 100 ng mL^{-1}. This is shown graphically in Figure 46 below.

Figure 46. Graph Demonstrating the Linear Relationship between the Concentration of Quinine Sulfate (ng mL^{-1}) and Fluorescence Response



Regression analysis using the least-squares method gave the equation $Y = 1.979x + 0.0903$, with a correlation coefficient (R^2) of 1, indicating a good linear dynamic range (*x* denotes the independent variable *i.e.* the concentration of QS, and *y* denotes the dependent variable *i.e.* fluorescence response). This was supported by visual inspection of the plot.

6.5.3.2 Limit of Quantification and Limit of Detection

The LoD and LoQ were determined by the analysis of samples of known concentration and the level measured by visual evaluation. The LoD (*i.e.* the smallest concentration that gave a measurable response[162]) was 0.1 ng mL QS. The LoQ was 1.0 ng mL^{-1}. It was the lowest concentration on the calibration plot, which could be quantitatively determined with reliable precision and accuracy *i.e.* CV = 4.3 %, and the range of accuracy was from 96.5% to 106%.

6.6. Experimental

A series of manipulations were carried out requiring dilution of QS, alternating with identical manipulations of WFI.

6.6.1 Batch Preparation

The batch preparation of WFI and QS was carried out in an isolator, closely simulating the dilution of two different drug solutions under actual working conditions. All personnel protective measures were undertaken. Overshoes, barrier hats, gloves and gowns were worn to simulate actual practice with the exception that an absorbent mat was not placed on the isolator base so that surface contamination on the base could be observed. Batches were initially prepared using the closed-system

(PhaSeal®) device, then the isolator was cleaned and the process was repeated using the 'open-system'.

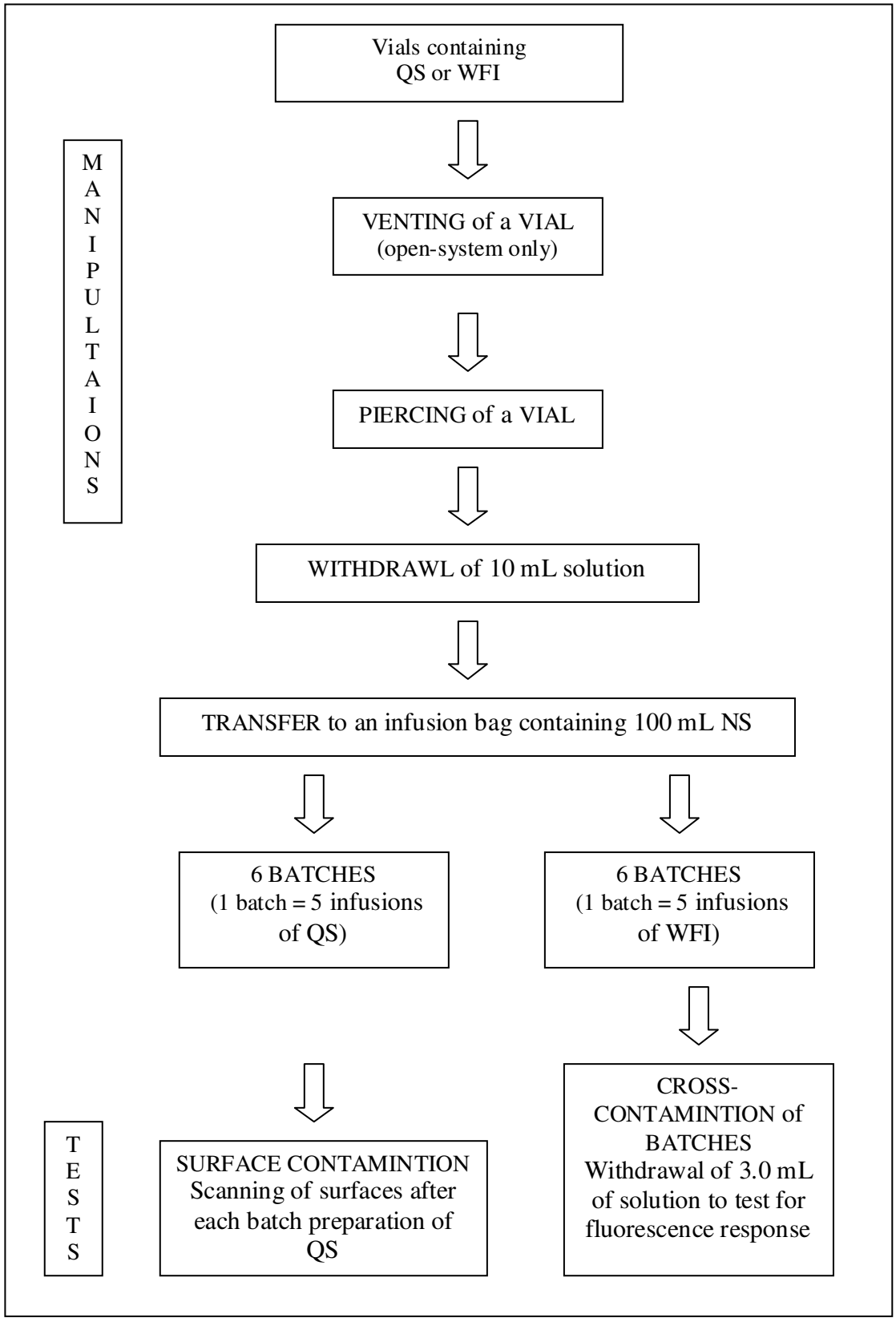
Using a 20.0 mL syringe, 10.0 mL of solution was drawn up from a 100 mL vial, and added to a Macoflex bag containing 100 mL of NS. This was repeated four times, to give a batch size of five infusion bags. Batches of infusions were prepared sequentially, alternating between batches of WFI and batches of QS. Six batches of each solution were prepared to give thirty infusions per solution. Operator technique was monitored throughout and any spillages or equipment failure was documented. This process is illustrated schematically in Figure 47 on the following page.

6.6.2 Monitoring of Surface Contamination

The manipulations were carried out under normal room lighting. After the preparation of each batch of QS, the lights were switched off and the surfaces of the isolator, hatch area and doors, gloves and sleeves in the isolator, and the infusion bags were scanned with UV light (365 nm). Areas of contamination were photographed using a digital camera. Prior to batch preparation using the open-system the isolator was cleaned and all surfaces were scanned to ensure an environment free from QS contamination.

The point was established during manufacture, when surface contamination of QS was first observed on the exterior surface of infusion bags, and in the isolator. From this point onwards, the frequency and level of QS surface contamination when using the open-system was compared when using the closed-system (PhaSeal®) device.

Figure 47. Flow Chart Showing the Process and Manipulations Carried out for the Preparation and Testing of QS and WFI Batches



6.6.3 Measurement of Product Cross-Contamination

All infusions from each batch of WFI prepared were retained and transported to the University of Bath to measure for the presence of any QS cross-contamination. A fluorescence spectrophotometer with a xenon power supply was used to measure fluorescence emission from the contents of the infusion bags. The excitation wavelength was set at 317 nm and the emission wavelength was set at 390 nm. To measure for QS, approximately 3.0 mL of solution was removed from each infusion bag and transferred into a cuvette for measurement of fluorescent intensity. The measurements were carried out in duplicate.

It was intended to establish the point during manufacture when the cross-contamination of infusion bags occurred for comparison with the closed-system (PhaSeal®) method.

6.7 Results

There were no spillages reported from the manipulations carried out using both methods for the production of all batches of WFI and QS. There were no problems with the closed-system (PhaSeal®) device except on one occasion during the preparation of a batch of QS when the injector was separated from the protector before the needle was fully retracted into the injector. This exposed the needle tip; immediately the needle was withdrawn back into the injector and secured with the safety latch. There were no droplets of contamination on the tip or released into the isolator that were visible to the naked eye. Batch preparation was temporarily halted and all areas were scanned with the UV lamp. There was no contamination observed on any of the surfaces concluding that this incidence had no effect on the study.

The same manipulations were carried out 30 times with WFI and QS using the closed-system (PhaSeal®) device. The isolator was cleaned and a clean environment confirmed prior to repeated manipulations using the open-system. The distilled water, in which the vials filled with QS were immersed in, showed no fluorescence emission response, confirming that the outside of the vials were clean. QS contamination was visible on the latex material of gloves used in the isolator. All surfaces scanned prior to the start of the study were negative for the presence of QS contamination.

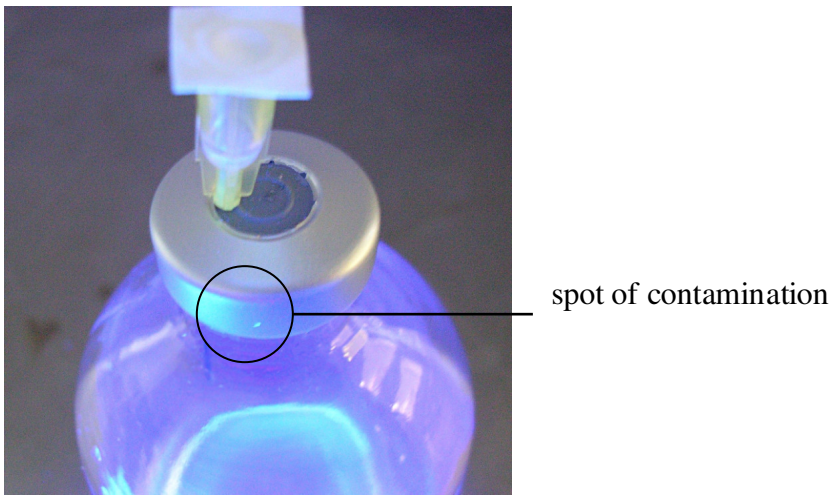
6.7.1 Surface Contamination

After the preparation of each batch of QS, surface contamination was made visible by scanning the surfaces with a UV lamp. Absorbance of QS was scanned for at a wavelength of 365 nm.

6.7.1.1 The Open-System

Surface contamination was clearly visible when using the open-system. The first occurrence of surface contamination was a small spot observed on the metal rim of a vial containing QS (see Figure 48 on the following page). This was observed after the production of QS batch 1, therefore it is not known at which point during the production of the batch 1 that it occurred. It was not a requirement of the study protocol to scan the vials. This contamination was observed inadvertently when scanning the batches after removal from the isolator. Its presence was considered important in this study and so it was photographed.

Figure 48. Image of a Vial with a Spot of Contamination on the Rim



The following incidences of surface contamination were observed only on the base of the isolator when using the open-system. No surface contamination was observed on the gloves, sleeves or hatches, or on the external surfaces of any of the infusion bags.

The first occurrence was in the centre of the base to the right-hand side of the area where batch manipulations were carried out. Contamination in the form of 4 large spots (see Figure 49 on the following page) were photographed. These were produced from the manipulations carried out to prepare 10 infusion bags of QS (2 batches).

The second occurrence of surface contamination on the isolator base was from the manipulations carried out to prepare 20 infusion bags (4 batches). Surface contamination was observed in two areas on the isolator base. Firstly, a ‘splash’ was photographed towards the left-hand corner of the base closest to the operator (see Figure 50 on the following page).

Figure 49. Image of Contamination after the Preparation of Two Batches of QS with the Open-System

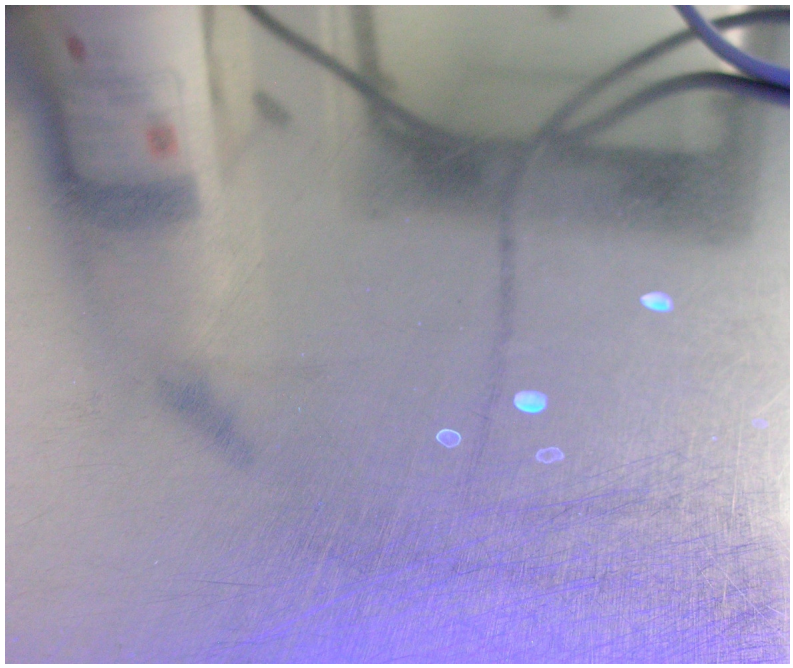
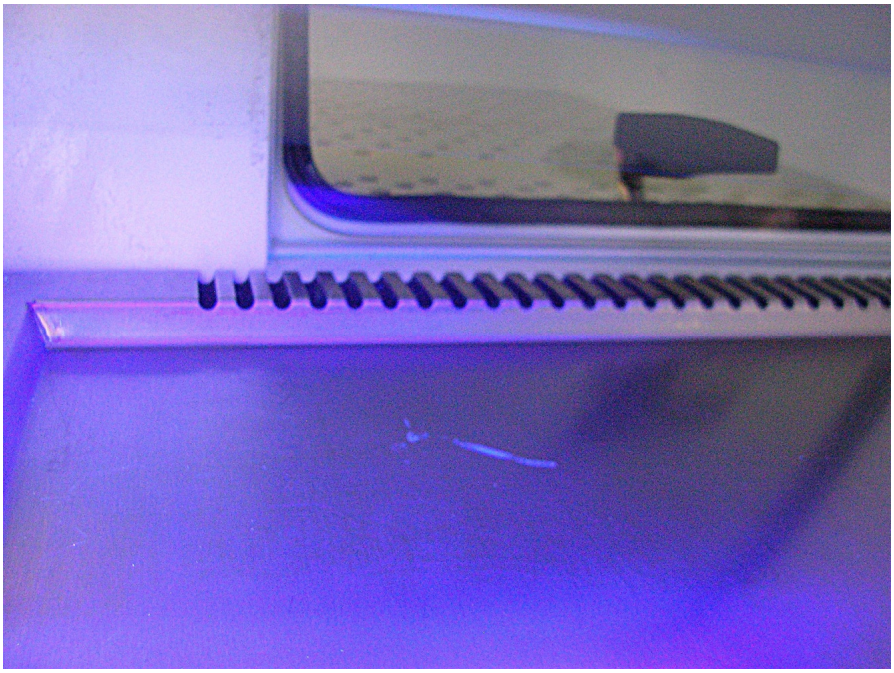
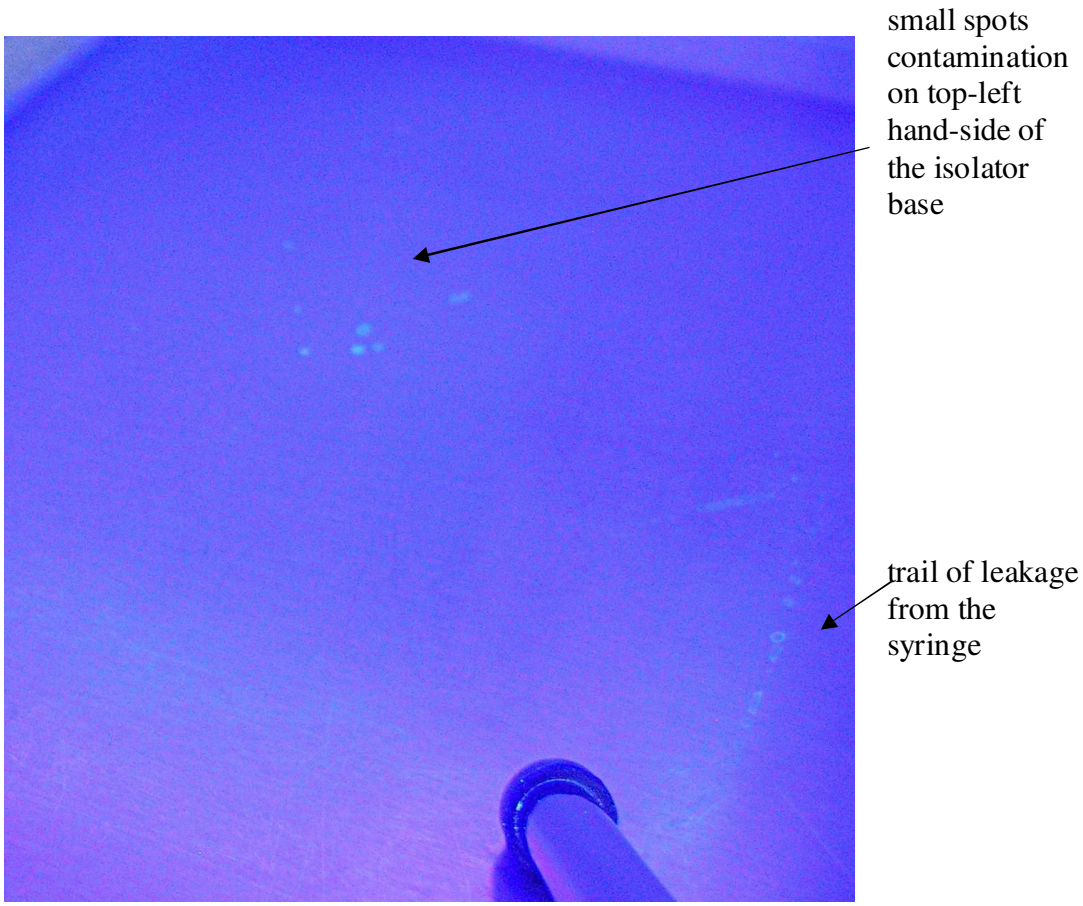


Figure 50. Image of a 'Splash' of Contamination after the Preparation of Four Batches of QS with the Open-System (left-hand corner of the isolator base closest to the operator)



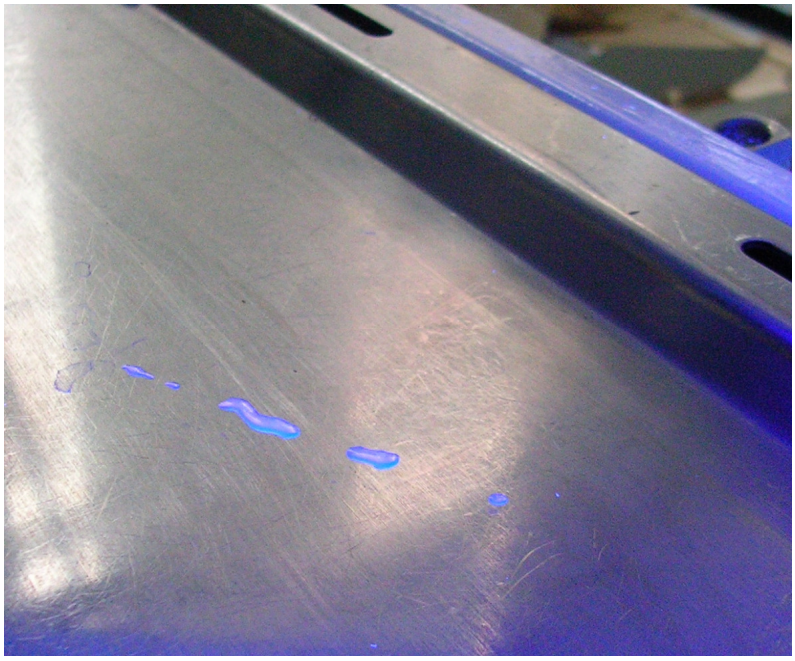
Secondly, contamination was observed in the top left-hand side of the base furthest from the operator; a collection of small spots are observed and a trail of leakage from the syringe (see Figure 51 below).

Figure 51. Image of Contamination after the Preparation of Four Batches of QS with the Open-System (top left-hand corner of the isolator base)



At the end of batch production *i.e.* after the production of 6 batches of QS (30 infusions), another leakage contributed towards the contamination observed on the isolator base. The leakage was a trail of large spots of contamination (see Figure 52 on the following page).

Figure 52. Image of Contamination after the Preparation of Thirty Batches of QS with the Open-System



No contamination was observed on any other surfaces in the isolator *i.e.* the walls and ceiling of the isolator chamber, sleeves, hatches or gloves, or on any of the infusion bags when prepared using the open-system.

6.7.1.2 The Closed-System

No contamination was observed on any of the surfaces in the isolator *i.e.* the base, walls and ceiling of the isolator chamber, gloves, sleeves or hatches, or on the external surfaces of any of the infusion bags when prepared using the closed-system (PhaSeal®) device.

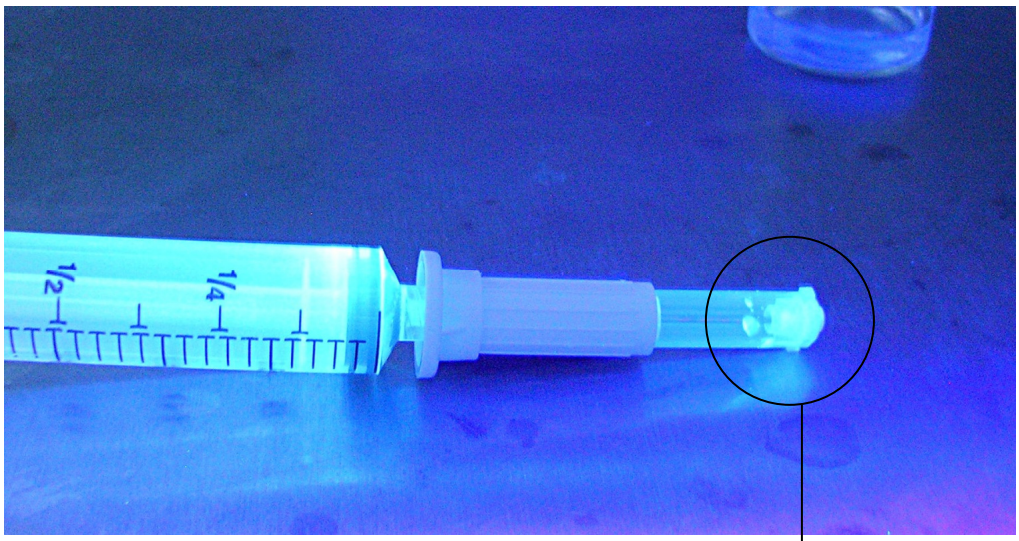
Figure 53 on the following page shows an image of an infusion bag with no external surface contamination. This infusion bag was diluted with QS using the closed-system (PhaSeal®) device.

Figure 54 on the following page shows an image of the injector component of the closed-system (PhaSeal®) device after batch preparation of QS. It was photographed

to show droplets of QS inside the injector contained behind the membrane of the PhaSeal® injector component.

Figure 53. Image of an Infusion Bag (0.01 mg mL^{-1} QS) with no External Surface Contamination

Figure 54. Image of a Closed-System (PhaSeal®) Assembled Syringe with QS Retained Inside the Injector



6.7.2 Product Cross-Contamination

All infusions from all batches of WFI prepared using both the open and the closed-system (PhaSeal®) methods were measured for fluorescence response. The initial concentration of the QS solution for dilution was 1.0 mg mL^{-1} and the sensitivity of the analytical detection method was 0.1 ng mL^{-1} of QS. Therefore, to identify a positive presence of QS in the infusion bags at the LoD of 0.1 ng mL^{-1}, 1/100,000th of a millilitre of QS cross-contamination would need to be detected. None of the infusions tested positive for fluorescence above the LoD of the analytical method *i.e.* there was no cross-contamination of the fluorescent marker QS.

6.8 Discussion

The manipulations carried out in this study as shown schematically in Figure 47 (page 269) were the piercing of a vial with a syringe/needle, the withdrawal of solution and its transfer to an I.V infusion bag. Repetitive dilutions carried out, alternating between the preparation of batches of WFI and batches of QS, simulated the sequential handling of two different drug solutions in the same isolator.

The first objective of this study was to identify surface contamination occurring in an isolator after the dilution of batches containing a highly fluorescent indicator. After the manipulation of each batch, surfaces were scanned and photographed under UV lighting to visualise any leaks and spillages.

A small spot of contamination was observed on a vial used to prepare the first batch of QS (Figure 48, page 272). This is likely to have occurred when removing the syringe needle from the vial. Otherwise, contamination was only observed on the isolator base, which was contaminated frequently when using the open-system. The first point of leakage occurred during the preparation of the second batch of QS, as contamination was observed at the end of production of batch 2. The 4 large spots

observed in Figure 49 (page 273) are possibly due to drips from the syringe needle when moving it towards the I.V. bag. The contamination observed in the lower left corner in Figure 50 (page 273) appears to be leakage from the removal of a syringe from the vial; drops from the needle of the syringe or leakage from the port of an I.V. bag when removing the syringe. The trail of smaller droplets in Figure 51 (page 274) observed in the top left corner after the preparation of batch 4 appears to be small droplets/aerosol released from the syringe, possibly when placing it down onto the base. The largest leakage was observed at the end of batch production after 30 manipulations had been carried out (Figure 52, page 275). This leakage was in the working zone of the operator and was visible to the naked eye. Nethertheless, when contamination is not visible it is difficult to avoid, thus it is easy to see how the inadvertent transfer of secondary contamination onto other surfaces may occur.

No surface contamination was observed on the gloves, sleeves, the surfaces of the isolator chamber or on any of the surfaces in the hatch. There were no pre-contaminating sources to influence the results of this study. All vials used in this study were clean and confirmed to be free from QS contamination prior to transfer into the isolator, eliminating a main pre-contaminating source. This would have prevented any initial glove contamination through contact with the vial, and subsequent contamination of surfaces touched by the gloves prior to batch preparation. Conclusively, any surface contamination found was as a result of batch preparation in the isolator.

When using the closed-system (PhaSeal®) device to prepare infusions, no leakage was observed after the preparation of any of the six batches. Scanning the surfaces at 365 nm showed no contamination on the gloves, sleeves, isolator chamber

surfaces, surfaces of the hatches or on the surface of the infusion bags. The function of the closed-system (PhaSeal®) device to contain contamination was further confirmed when an injector was scanned after use (Figure 54, page 276). QS contamination was observed inside the end of the injector, demonstrating that contamination would have been released into the environment either during the piercing or the withdrawal of QS solution from the vial. This contamination was contained safely inside the injector, fulfilling its role as a closed-system and preventing the release of contamination into the outside environment. Furthermore, the components of the closed-system (PhaSeal®) device are disposable and it does not need to be disassembled from the vial or syringe. Therefore, the benefits of containment would also continue through the disposal process.

It would be expected that the isolator base would be one of the main areas contaminated in an isolator when carrying out dilutions. The first contamination event occurred early on *i.e.* during the preparation of the second batch and increased with batch preparation, contaminating different areas of the isolator base around the preparation area. The surface contamination made visible in this study supports findings that the traditional open-system has the potential to allow the release of contamination into the surroundings. It also supports the effectiveness of the closed-system (PhaSeal®) device in preventing the release of contamination when the dilution of batches was carried out.[32]

The second objective of this study was to determine if the cross-contamination of batches does occur after the sequential dilution of different solutions in an isolator. A method using the native fluorescence emission of QS was sensitive to measure and

quantify cross-contamination as low as 1.0 ng mL^{-1} in the infusions of batches prepared with WFI. Cross-contamination may not have occurred, or it may have been present in lower concentrations but was not detectable. Mass-spectroscopy in conjunction with either gas-chromatography or liquid-chromatography is a more sensitive method of detection. However, a more sensitive method than the method using the native fluorescence of QS in this study (LoD = 0.1 ng mL^{-1}) could not be found when searching the literature. The references retrieved were methods for the quantification of QS from biological samples. The methods were solid-phase extraction and gas-chromatography/mass-spectrometry,[194] capillary liquid-chromatography with native fluorescence,[195] and chemiluminescence,[196] reporting LoDs of 12.2 ng mL^{-1}, 5.0 ng mL^{-1}, and 1.5 ng mL^{-1}, respectively. It is expected that the presence of contamination on surfaces would increase the possibility of batch cross-contamination occurring. However, contamination observed on the base of the isolator did not influence the cross-contamination of batches, suggesting that the cross-contamination of batches does not occur, certainly for this size of batch production, and does not pose a risk in isolators.

6.9 Conclusion

This study visually confirms the release of contamination onto surfaces in the immediate environment when carrying out dilutions using the open-system, and the containment of contamination when using the closed-system (PhaSeal®) method. When using the open-system the base of the isolator was the surface contaminated the most highly and frequently. There was no surface contamination observed on any surface in the isolator when using the closed-system (PhaSeal®) device.

No cross-contamination of QS was detected in any of the infusions prepared using the open-system or when using the closed-system (PhaSeal®) method.

Consequently, it was not possible to compare the two methods or assess the effectiveness of the closed-system (PhaSeal®) device in reducing or preventing the cross-contamination of batches after preparation in the same isolator, although intuitively the risk of this occurring should be reduced with the closed-system (PhaSeal®) device.

7. Concluding Discussion and Conclusion

7.1 Concluding Discussion

Under the Health and Safety Act 1974 and the Management of Health and Safety at Work Regulations 1999 the employer has a duty to protect the health of its employees. The simulated compounding of cytotoxic drugs in an isolator under UK standards of practice (Chapter 5) demonstrated that immediate surfaces in the isolator and the finished product become contaminated from this process. This was supported where surface contamination was visually observed on the isolator base (Figures 48 - 52, pages 272 - 275) when using the open-system for the preparation of syringe doses. Surface contamination has the potential for inhalation, glove penetration and subsequent dermal contact. It may also be potentially transferred to other environments, posing a risk to personnel working in the clean-room and nurses who would administer the infusion dose.

Pharmacy technicians and assistants are the main subjects of occupational exposure in centralised hospital pharmacies. The lack of concern from pharmacists managing ASUs (Section 2.4.3.1) suggests that they do not appear to be aware of their responsibilities. They may be more concerned if it was the role of the pharmacist to carry out cytotoxic manipulations. COSHH regulations specify that regular monitoring of the work environment must be undertaken when employees are exposed to substances hazardous to health. Many hospital pharmacies do not have the facilities to measure the low levels of contamination that are likely to be present. Further, measuring for cytotoxic surface contamination is limited and no airborne monitoring of cytotoxic contaminants is being carried out (Section 2.4.3.7). This is of great concern, as nearly half of the isolators in use re-circulate air that may be contaminated back into the clean-room.

Not all drugs can be measured at sensitive levels, as a result, only a fraction of cytotoxic drugs have been monitored in the workplace. It is important to measure more than one marker drug as contamination may be influenced by various factors, particularly the nature of the product *i.e.* volatility, pharmaceutical form - liquid or powder, different preparation modalities and the quantities of drug handled.[189]

The levels of multi-drug surface contamination measured in this thesis (Section 5.5) were influenced by the chemical properties of the marker drugs used. CP does not possess a strongly absorbing chromophore, unlike EPI and MTX', thus the same level of sensitivity could not be achieved for all three drugs in one single recovery method (Chapter 3). CP contributed more cumulative contamination to isolator surfaces during the Baseline period using the open-system. This is of great concern, as there are no safe established levels, and CP is one of the most potent cytotoxic drugs. The significance of including CP was as a marker drug for comparative measurement with other studies of the closed-system (PhaSeal®) device.

The closed-system (PhaSeal®) device was accepted and preferred by pharmacy technicians to the open-system (Tables 51 - 53, pages 238 - 240). The projection of data (Table 58, page 256), demonstrated how effective such intervention would be in reducing the potential long-term risk to surface contamination. There are other devices available commercially claiming a closed-system (Section 1.9) which should be investigated and compared. However, the NIOSH definition of a closed-system device gives no performance standard or test system for such a device, or acceptable levels of contamination when using the device.[4]

Testing marker drugs with different stability profiles from different chemical classes (Chapters 4 and 5), demonstrated the need to approach and treat cytotoxic

contamination of different drugs selectively. Decontamination procedures that are effective for one drug may not be so effective for another, and a more stringent application may be required for more stable drugs.

One participant of the questionnaire (Chapter 2) expressed that "the research objectives must be to compare different cleaning procedures and establish which are the most effective that can be recommended to users". The products chosen for decontamination primarily have maintenance of the bio-burden level inside the isolator in mind, to protect product sterility (Section 2.4.3.5). It should be considered that surface contamination may consist of multi-drug chemical contamination, as many different cytotoxic drug batches are handled simultaneously inside an isolator, increasing the risk of surface cross-contamination (Section 5.5.1 and 5.5.3). The solvent basis of the formulation of the cleaning agent should be considered carefully, and a combination of agents may be required to cover complete removal.

The pH-detergents, cleaning agents, IMS and WFI were effective in removing cytotoxic contamination on a small scale (Table 27, page 166). However, the cleaning agents, WFI and IMS, when combined in a cleaning protocol (Section 5.4.6 – Cleaning Procedure) were not always effective in removing cytotoxic contamination in an isolator (Tables 48 – 50, pages 234 - 236). In some incidences, an increase in contamination was observed after cleaning, on other occasions a reduction or no change in surface contamination was observed. The reason for the ineffectiveness of this procedure when put into practice may be that the cleaning action was not consistent in pressure or in coverage of the areas, or the contamination was redistributed by the wiping action onto other areas of the surface with which the wipe may have had contact.

The only degradation effect observed by the pH-detergents and cleaning agents, was on DOX, by the alkaline detergents. Limited success was observed when applied to drugs representing the other classes *i.e.* the alkylating agents (CP) and the antimetabolites (5-FU). A longer reaction time may have lead to eventual degradation, but ideally, the detergents would need to act within a quick contact time. The pH-detergents applied in this study were formulated for specific target soil (biological control). It may be necessary; to vary the formulation to target the more chemically stable structures of 5-FU or CP.

Applying a safe controlled gassing/fumigation of the internal workspaces of an isolator enables the even decontamination of areas otherwise inaccessible manually.[110] The advantages of gaseous hydrogen peroxide versus liquid hydrogen peroxide were observed experimentally (Section 4.5.4). It can be appreciated from the VHP® exposures carried out in this study that an increase in exposure time can make a significant difference. The duration time of the most effective VHP® cycle in the decontamination studies (Cycle 3) was 4 hrs and 11 mins. Fumigation/gassing methods are more time consuming and the cycle time would be too long to apply in-between batch preparation or sessions during a working day. This cycle would be more suitable for application at the end of a working day, and left to run overnight to decontaminate an isolator from biological and chemical contamination.

The questionnaire demonstrated that hospital pharmacies in the are already preparing MABs in a separate isolator if available (Section 2.4.3.4), but this may not be an option for all pharmacies. The effectiveness of the closed-system (PhaSeal®) device in preventing cross-contamination could not be ascertained in this study, as the cross-contamination of infusions was not observed in the batches prepared (Section

6.7.2). Surface contamination recovered from the isolator (Section 5.5.1), the positive occurrence of external surface cross-contamination on batches of different drugs prepared previously in the same isolator (Section 5.5.3), and the ineffectiveness of the cleaning procedure (Section 5.5.6) would be expected to increase the probability of solution cross-contamination occurring. The effectiveness of the closed-system (PhaSeal®) device in reducing both surface and surface cross-contamination in the environment would reduce the potential risk of drug solution cross-contamination occurring.

The supply of pre-contaminated vials has been suggested to be a significant factor in influencing cytotoxic surface contamination results in previous studies, and was suggested to be as a result of the high levels of CP recovered in the clean-room in this present study (Table 47, page 232).[26;126] Surface contamination was found on vials in 2 out of 7 cases when measured in ASUs (Table 4, page 74). The receipt of pre-contaminated vials was also a criticism, which was expressed by some participants of the questionnaire. The consensus was that it is the manufacturers' responsibility to ensure the supply of cytotoxic contamination-free vials, and busy hospital pharmacies should not have the additional task of cleaning every vial, supplied to the pharmacy. Methods have been published addressing a simple vial cleaning procedure which could be adopted by manufacturers.[45] It may be suggested that manufacturers are required to provide a written guarantee that the vials they supply have been cleaned according to a validated process and are contamination-free. Hospital pharmacies should then only purchase from these manufacturers, encouraging other manufactures to follow suit.

7.1.1 Implications of the Project for Practice

This study has several implications for the practice of cytotoxic drug reconstitution and manipulation, which would gain an overall improvement in safety for those who manipulate these drugs. It should inform the managers of hospital pharmacies in the UK of the levels of contamination that can occur in an isolator and in the immediate environment as a result of the compounding of cytotoxic drugs. For staff pursuing a short or long-term career of working with cytotoxic drugs, this study shows the amount of surface contamination they would have the potential to be exposed to, unless changes in practice take place.

Current practice should reassess the agents that are being used for cleaning and take into consideration chemical decontamination in addition to biological decontamination. It should be considered that one agent will not be suitable for all cytotoxic drug contamination, and cleaning agents should be chosen based on the physical chemistry of the contaminating drug. Selection would be based on whether the drug contamination is water-soluble, the pH at which the contaminating drug would be ionised and therefore be solubilised and removed from the surface by the cleaning agent. This should encourage the adoption of standard, validated techniques to minimise occupational exposure to chemical contamination from cytotoxic drugs.

The results from the studies carried out in this thesis have presented additional methods which should be implemented in hospital pharmacies to further reduce the levels of cytotoxic surface contamination which personnel may potentially be exposed to. The implementation of these methods would also reduce contamination on the external surfaces of the finished product, benefiting personnel working in clinics or on hospital wards. Action taken by manufacturers to use validated cleaning procedures and supply cytotoxic contamination-free drug vials

may also significantly reduce the levels of pre-contamination in the pharmacy environment.

Use of a closed-system device such as the PhaSeal® device to prepare syringe batches and infusions in an isolator would significantly reduce levels of surface contamination. However, this would incur a cost (~$10 a dose),[197] and financial constraints may be a limiting factor for its implementation into NHS hospital pharmacy practice. These costs and risks need to be considered by hospital Trust managers.

7.1.2 Key Limitations of the Work

A large number of different drug infusions of varying cytotoxicity are prepared in hospital pharmacies; it was therefore practical to select a small number of representative marker drugs for the purpose of this work. They were chosen for the following reasons; they are compounded in relatively large amounts, represent different chemical classes of cytotoxic drug, and are relatively well established. Nethertheless, it must be considered that physical and chemical properties and metabolic pathways are different for each drug and the determination of a larger number of compounds would provide a wider representative for risk assessment.

The decontamination study was a pilot study and demonstrated visible cytotoxic contamination to be removed easily from a small surface. In practice, the surfaces of the interior of the isolator to be decontaminated would be a much larger area, manufactured of several materials and be of a more complex topology.

The compounding of batches of cytotoxic drugs was carried out in an isolator situated in a clean-room environment in an academic setting. The study simulated actual practice, which would be carried out in a hospital environment. One of the important aspects of this situation was that it enabled the batches of cytotoxic drugs

prepared, which would otherwise be administered to the patient, to be retained for sampling.

Prior to the start of the study, the isolator and the clean-room environment were clean *i.e.* free from cytotoxic contamination. Although ideal for research purposes, this would not be true of an isolator used in a busy hospital environment for the multi-drug batch preparation of cytotoxic drugs and other cancer treatments. It is therefore likely that the results of this simulated study may be an under-estimation of the amount of surface contamination that would actually be present on the equivalent surfaces in a hospital pharmacy environment. The situation did however enable the compounding of the three marker drugs to be restricted to one isolator, eliminating the risk of interference of the drug assays from other co-prepared drugs.

7.1.3 Future Work

Further investigations into VHP® exposure cycle parameters would be essential to develop decontamination cycles to achieve the chemical breakdown of cytotoxic drugs other than the marker drugs investigated in this thesis. VHP® injected under vacuum would achieve direct contact and greater penetration of VHP® into the target. The vacuum is created prior to injection and the number of pulses per cycle can be controlled, thus increasing and controlling the amount of VHP® in an exposure. Under vacuum, the impediment of air is removed and the enclosed area fills with VHP® only. This method has already been demonstrated to be effective against resistant spores, which were resistant to oxidation by VHP® under normal atmospheric conditions.[186] This is a more rapid process than the VHP® exposure cycles carried out in this thesis. The samples can be removed from the exposure vessel at certain time intervals, without the aeration time-consuming phase of the

cycle. Exposure and removal of several samples at certain time intervals would allow the rate constant of the reaction to be calculated and the optimal conditions of temperature and VHP® exposure time for the degradation of the drug to be determined. The cycle conditions could be optimised to cover a wide spectrum of drugs. 5-FU, a drug that is unsusceptible to degradation by VHP® may be applied as the worse case marker for the optimisation process. This could be determined for DOX and EPI and extended to daunorubicin, and other cytotoxic drugs likely to undergo oxidation *i.e.* mitoxantrone, bleomycin etoposide, mitomycin C, teniposide, MTX, cisplatin.[24] It could also be determined for CP and extended to other members of the same chemical family *i.e*. IFOS, although parameters that are more stringent may be required. In addition, it may be extended to biological treatments for cancer which are also prepared in hospital pharmacies and carry the risk of biological cross-contamination *e.g.* BGC, a microorganism and immunotherapeutic agent, prepared by many pharmacies for the treatment of superficial bladder cancer.[191] The contribution to toxicity of any degradation products would need to be investigated thoroughly and identified. Methods that are more sensitive would also be required if further degradation effects of VHP® were to be further investigated. Sensitivity may be achieved by using liquid-chromatography mass-spectroscopy, or HPLC coupled with fluorescence detection for DOX and EPI.

The preliminary decontamination studies with the pH-detergents and cleaning agents also leaves open the possibility for future work. The cleaning system used should be explored to find cleaning agents more suitable to the chemistry of EPI, MTX and CP. The alkaline detergents, which were effective in the degradation of DOX, may also be effective for EPI. These should be investigated on a larger surface

area and on surfaces of different material *i.e.* stainless steel. This may include increasing and ultimately optimising, the ratio of detergent to surface area.

The cleaning agents that were effective in removing cytotoxic surface contamination on a small scale should also be extended to larger surfaces. The role of other cleaning agents that are used commonly in ASUs and available formulations containing sodium hypochlorite (5.25%) should also be investigated for the removal and degradation of cytotoxic contamination.

There are other devices available commercially claiming to be closed-systems, which are designed for cytotoxic containment. These claims should be assessed in similar studies as investigated in Chapter 5 of this thesis. Testing should be carried out in a clinical setting or simulating actual practice to provide a valid comparison between devices, and ascertain whether the system used under practice conditions is closed or not. Scientific proof that the device is closed must be published in peer-reviewed journals before the integrity and validity of the device can be ensured. Other similar devices would make the market more competitive and possibly reduce the financial burden.

7.1.4 Horizons

As the number of cancer cases increase, the amount of chemotherapy doses prepared and administered will also increase, resulting in an even greater potential for occupational exposure of health-care professionals. Novel drugs and therapeutic approaches are still being discovered and introduced into clinical practice. Some are analogues of agents that have already proven to be effective chemically and pharmacologically, some are natural products isolated from plants or microorganisms and others are designed based on biological approaches *e.g.* potential inhibitors of enzymes or essential cellular components. The conventional cytotoxic drugs are not

specific to cancer cells. As cellular pathways become more understood, future cytotoxic drugs will be developed based on more rational and mechanism-based approaches. There have already been moves towards therapies that target cancer cells specifically. Currently, the major classes of targeted therapies, which reflect these new directions and mechanisms of action are the MABs, tyrosine kinases and cell-cycle specific agents.[198;199] Many of the tyrosine kinases that have emerged are for oral administation.[198]

The upward trend in the number of oral chemotherapy agents, which are suitable for home administration, is likely to continue.[200] Although this route of administration has advantages and disadvantages, patient preference is likely to be towards the oral route for convenience, the preference of administration in the home setting, or due to the fear of needles.[198] The use of oral chemotherapy could potentially reduce the use of healthcare resources and ancillary support personnel such as nurses and technicians.[198] This would engage patient involvement outside the healthcare environment and non-adherence by the patient may be an issue. Reliance from the healthcare practitioner on educating the patient in managing their oral chemotherapy would be required. It has been suggested that guidelines specific to safe handling of oral chemotherapy in the non-conventional setting *i.e.* the home environment are developed.[201]

There is little information available as to how hospital pharmacies should manipulate viral and genetic material. It has been acknowledged that information is lacking as to which decontamination procedures should be used for viral products,[202] and suggested that there need to be separate facilities for the manipulation of these products.[203] This could also be a key role for closed fluid-transfer systems.

The risks of drug toxicities may be justifiable to patients undergoing treatment for a life-threatening disease but such risks, even at a reduced level are not acceptable to healthcare professionals involved in drug preparation or administration. It should be recognised that technicians may be facing a long career of exposure to cytotoxic drugs and novel drugs developed in the future of unknown toxicity. Future breakthroughs will almost certainly occur and influence practice in this area. Instead of preparing each dose on a bespoke basis after receipt of the prescription, dose-banded syringes pre-filled by industry may be supplied. The prescribing of standard doses prepared based on calculations from the body surface area of patients would require no further manipulation in the pharmacy.[203] Novel formulations are already entering into clinical trials and some are available commercially. These changes will make prediction of future occupational risks very difficult to define.

7.2 Conclusion

Isolators are used in hospital pharmacies for the compounding of cytotoxic drugs. In the facilities which responded to the survey, 17 out of 42 (40.5%) isolators are ducted internally. They are decontaminated according to a protocol, which considers cleaning and disinfection. ASU managers of these facilities were not profoundly concerned regarding cytotoxic contamination. The purpose of the decontamination products used was to address the removal of biological and not chemical cytotoxic contamination. The products used for decontamination of the isolator were limited to proprietary products, mostly supplied by the same manufacturer. These decontamination procedures were developed from information provided by national guidelines and books, quality control and assurance, other ASUs and manufacturers' advice. The effectiveness of these procedures for the removal of cytotoxic contamination were not often validated or tested due to the lack

of on-site equipment and expertise to analyse cytotoxic contamination. The current protocol used most widely for decontamination of the isolator would be to clean using IMS spray with dry wipes. A detergent/liquid biocide *i.e.* neutral detergent or QACs/biguanide or QACs/stabilised chlorine dioxide may also be used. For disinfection, again alcohol and/or detergents/liquid biocides *i.e.* QACs, QACs/stabilised chlorine dioxide, QACs/biguanide would be used.

Decontamination methods can be used to reduce, if not eliminate, the risk posed by cytotoxic drugs to the operator and environment. The extent of risk reduction or elimination will vary depending on the structure of the drug, but may be extended to drugs of the same family. Alkaline-based detergents CIP 100, CIP 150 and Criti-Klenz have resulted in the breakdown of DOX, and oxidation by VHP® has resulted in the breakdown of DOX and EPI. CP was susceptible to degradation by VHP® under the more stringent oxidative conditions, but 5-FU was less susceptible. VHP® decontamination may be considered for drugs, which are prone to oxidation such as the anthracyclines, and also for CP, for routine biological and cytotoxic contamination within isolators.

The compounding of cytotoxic drugs contaminates surfaces within the isolator when using the open-system. The first contaminating event occurs rapidly. Once present, surface contamination in the isolator was resistant to removal by cleaning agents (Klercide CR-B and Klerclean neutral detergent) used currently in ASUs. Isolators have the capability of protecting immediate areas from multi-cytotoxic drug surface contamination when used in conjunction with a closed-system (PhaSeal®) device. The closed-system (PhaSeal®) device evaluated in this study exerted control on contamination in the surrounding environment *i.e.* in an isolator.

This was true for two drugs (EPI and MTX), novel to usage with the closed-system (PhaSeal®) device, and was supported by the results for CP which are in accordance with previous studies found by other investigators.[20;62;67;124;154] Operators of the closed-system device preferred it to the traditional open-system method.

If no further changes occur or no further methods are introduced to protect the worker, implementing a closed-system (PhaSeal®) device over 1 year or 40 years would significantly reduce the potential risk of occupational exposure to pharmacy personnel from surface contamination. This risk reduction would not only include personnel who work in the isolator and clean-room, but those who work in clinics or on wards.

The risk of drug cross-contamination of the injection solution from batches prepared previously during the same session was not evident. For this reason, the effectiveness of the closed-system (PhaSeal®) device in reducing solution cross-contamination could not be determined.

High levels of surface contamination were recovered from surfaces in the clean-room. The high levels of CP were influenced possibly by the supply of pre-contaminated vials. Pharmaceutical manufacturers should be encouraged to improve their decontamination procedures and to guarantee the supply of vials free of cytotoxic contamination.

Universally, pharmacy managers of hospital pharmacies should recognize their responsibilities and use decontamination procedures that have been validated and proven to remove not only biological contamination but also cytotoxic contamination. These procedures should be validated and defined intervals of

environmental monitoring implemented. Only then, can levels of environmental contamination be established and a safe level of exposure be determined.

The risk posed by cytotoxic drugs to the operator and the environment may be reduced, if not eliminated by considering additional approaches. Firstly, the application of effective decontamination methods; and secondly, by using a closed-system drug transfer device such as the PhaSeal® device in a controlled environment, such as in an isolator.

7.3 Papers and Abstracts

The listed below can be found on pages 298 to 311:

- Journal of Oncology Pharmacy Practice Publication (2006) 12; 95 - 104. Studies on the decontamination of surfaces exposed to cytotoxic drugs in chemotherapy workstations.

- Hospital Pharmacy Europe Publication (2007) 33; 17 - 19 Safe handling of cytotoxics.

- ISOPP Abstract - Studies on the Decontamination of Cytotoxic Drugs on Surfaces in Chemotherapy Workstations. Journal of Oncology Pharmacy Practice (2006) 12; 1; 34.

7.4 Conference Presentations

The listed below can be found on page 312:

- ISOPP Poster - Studies on the Decontamination of Cytotoxic Drugs on Surfaces in Chemotherapy Workstations.

7.5 Award

The listed below can be found on page 313:

- ISOPP Travel Award.

J Oncol Pharm Practice (2006) 12: 95–104

Studies on the decontamination of surfaces exposed to cytotoxic drugs in chemotherapy workstations

Sarah Roberts, Bsc[1]
Nancy Khammo, PhD[2]
Gerald McDonnell, PhD[2]
Graham J Sewell, PhD, BPharm[3]

Objective. The aim of this study was to examine the removal and deactivation of cytotoxic contamination from surfaces of a pharmaceutical isolator workstation.

Methods. Three marker cytotoxic drugs were evaluated in three phases using decontamination technologies currently available in the pharmaceutical and healthcare environments. Phase I investigated the physical removal of contamination by detergents. Phase II and III investigated the effectiveness of detergents and Vaporised Hydrogen Peroxide (VHP®) in degrading cytotoxic drugs, respectively.

Results. 5-Flurouracil, doxorubicin and cyclophosphamide were removed from a surface by wiping with detergents. VHP® and alkaline detergents caused degradation of doxorubicin. The observed effect with detergent cleaning was pH dependent, but neither of the technologies applied had any effect on the chemical stability of 5-flurouracil and cyclophosphamide under the conditions tested. ***J Oncol Pharm Practice (2006) 12: 95–104.***

Key words: cyclophosphamide; cytotoxic; decontamination; degradation; doxorubicin; 5-flurouracil; vaporized hydrogen peroxide

INTRODUCTION

Chemotherapy is the only systemic treatment modality for cancer. However, cytotoxic drugs are not selective for cancer cells, but also effect the growth and reproduction of healthy cells. During the preparation of cytotoxic infusions, a variety of drug manipulations are performed, resulting in the generation of aerosols and droplets, which are known to contaminate the areas in which they disperse into, including isolators and surrounding surfaces.[1–9] This increases the risk of occupational exposure to these drugs, for which the health effects are well documented.[10–13]

The effectiveness of removing cytotoxic residues from surfaces during cleaning is not often considered. Ideally, removing cytotoxic contamination should involve the physical removal of drug contamination from a surface and drug breakdown into less toxic compounds. Cytotoxic drugs represent a diverse range of chemical structures, and no single agent is known to deactivate all the cytotoxic drugs currently used. Therefore, decontamination is limited to the mechanical removal from a non-disposable surface to a disposable surface, ie, by wiping the working surface with a cleaning agent. The National Institute for Occupational Health and Safety (NIOSH) recommends that all surfaces are decontaminated according to a protocol, which includes an appropriate deactivation agent if available.[14] The agent used should preferably demonstrate removal/breakdown

From the [1]Department of Pharmacy and Pharmacology, University of Bath, Bath, UK; [2]Steris Ltd, Basingstoke, Hampshire, UK; [3]Department of Pharmacy, Kingston University, Kingston-Upon-Thames, Surrey, UK

Address correspondence and reprint requests to Professor GJ Sewell, Department of Pharmacy, Kingston University, Penrhyn Road, Kingston, Surrey, KT1 2EE, UK
E-mail: g.j.sewell@kingston.ac.uk
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of biological and chemical contamination.[14] Currently, the most prevalent practice consists of surface wiping with water, with or without a detergent, with thorough rinsing, followed by wiping with 70% alcohol.

In 1985, the International Agency for Research on Cancer (IARC) included cytotoxic drugs in its program for the treatment of contaminated waste,[15] and oxidation was suggested as a method for degrading these compounds. Studies carried out to investigate the efficacy of oxidizing agents used in hospitals showed sodium hypochlorite (5.25%) was >99.96% efficient at degrading several drugs, including cyclophosphamide and doxorubicin. Liquid hydrogen peroxide (30%) was also successful.[16,17]

The aim of this study was to investigate the safe decontamination of areas used for the dispensing of cytotoxic drugs, by evaluating systems that remove and deactivate cytotoxic contamination from the surface of an isolator workstation. Decontamination may be defined as the use of physical and/or chemical means to render a surface or item safe for handling, use or disposal. This can refer to both chemical and biological decontamination, which are important in the safe dispensing of cytotoxic drugs. Decontamination is generally a combination of cleaning (to physically remove surface contamination) and disinfection/sterilization (which are anti-microbial processes).

The effects of two decontamination technologies currently in practice, ie, vaporised hydrogen peroxide (VHP®) and liquid detergents, on three marker cytotoxic drugs were evaluated.

VHP® is an odourless, colourless gas that is produced by vaporization of liquid hydrogen peroxide to give a mixture of hydrogen peroxide and water vapour. The systems used to generate VHP® were based on the delivery of a 'dry' (or non-condensed) hydrogen peroxide gas within a given area.[18] In this gaseous state, the biocidal and surface compatibility properties are markedly superior to liquid hydrogen peroxide.[19] The liquid detergents were formulations scientifically designed for the removal of specific material, eg, protein- or mineral-based material from various surfaces, and are in current use for various hospital and pharmaceutical applications.

The decontamination of cytotoxic drugs on surfaces using the decontamination technologies currently available was investigated. The aim was not only to investigate the removal of the drugs, but also the effects of detergents and biocides tested in the interaction or break-down of the drugs into safe by-products. This was evaluated in three phases:

Phases I and II investigated the effectiveness of aqueous-based detergents across a pH range of 1.7–13.2, as cleaning agents, which could be incorporated into a cleaning protocol;

Phase I – The physical removal of contamination from a surface by wiping with the detergents;
Phase II – The ability of the detergents as deactivating agents.
Phase III investigated the ability of VHP® to degrade the drugs on an inert surface by oxidation.

The three marker cytotoxic drugs used in this study were from different drug classes: 5-fluorouracil (5-FU, an anti-metabolite), cyclophosphamide (CP, an alkylating agent) and doxorubicin (DOX, an anthracycline antibiotic). These drugs are commonly used in the treatment of cancer and their sensitivity to degradation by various mechanisms is well documented.

MATERIALS

5-FU (250 mg/10 mL) and DOX (2 mg/mL) were obtained from Mayne Pharma Plc (Leamington Spa, UK); CP (500 mg) was obtained from Pzifer Ltd (Sandwich, UK). The test surface used in this study was made 'in-house' from the barrel of polypropylene syringes (Beckton and Dickinson, UK). Centrifuge (15 mL) tubes were obtained from Sarstedt (Leics, UK). Liquid hydrogen peroxide (Vaprox®), VHP 100P generator (Serial No. 0135103–29), flexible walled isolator, chemical indicators (Chemdi VHP code: NB305), and detergents (CIP 100, CIP 150, Criti-Klenz, Renu-Klenz, NpH-Klenz, Cage-Klenz 250, CIP 200 and CIP 220) were supplied by STERIS Corporation (Basingstoke, UK). Acetonitrile, disodium hydrogen orthophosphate, methanol, potassium dihydrogen orthophosphate were purchased from Fisher Scientific (Leics, UK). Ammonium sulphate, perchloric acid, sodium chloride and sulphuric acid were purchased from BDH (Poole, UK). Normal saline (NS) and sterile water for injections (WFI) were obtained from Baxter (Newbury, UK). Industrial methylated spirit (IMS) was obtained from Shield Medicare (Surrey. UK). All chemicals and reagents used for high-performance liquid chromatography (HPLC) were of analytical grade or (HPLC) grade. Hydrogen peroxide detection tube and hand held pump (Accuro®) were obtained from Dräeger (UK). The HPLC system comprised of an isocratic

constaMetric 3200 pump (LDC analytical), autosampler 851-AS (Jasco) and a variable wavelength UV detector (Applied Biosystems). Data analysis was performed using Prime software, version 4.2.0 (HPLC Technology, Herts, UK). HPLC columns were purchased from Phenomenex (Cheshire, UK) and HPLC Technology (Herts, UK).

METHODOLOGY

Cytotoxic drug preparations, surface coating of test surfaces and Phase I and II of the study were carried out in a class II biological safety cabinet (BSC). Exposure of the drug to VHP® was carried out in a flexible-walled isolator.

Choice of diluent

All three marker drugs were reconstituted or diluted in two common clinical diluents; WFI, NS and the HPLC buffer specific for each drug assay, ie, phosphate buffer (0.01 M, pH 7.0) was used for 5-FU; ammonium sulphate buffer (0.01 M, pH 3.5) was used for CP; and sodium chloride (0.01 M, pH 2.25) was used for DOX, to study any pH effect. Phase I, which involved removal by wiping was carried out on the drugs diluted in WFI and NS. In Phase II tests, WFI was used, and for Phase III, all diluents were used. Method validation was carried out with all diluents to take into account any variation in HPLC detector response.

Drug reconstitution

All cytotoxic drugs were diluted or reconstituted to the working concentration. 5-FU (25 mg/mL) was diluted in all three diluents to give a final concentration of 5 mg/mL, and 10 µL was transferred onto the test surface. The final concentration for assay was 10 µg/mL. CP (500 mg) was reconstituted in all three diluents to give a final concentration of 20 mg/mL, and 20 µL was transferred onto the test surface. The final concentration for assay was 400 µg/mL. DOX (2 mg/mL) was diluted to 1 mg/mL in all three diluents, and 10 µL was transferred onto the test surface. The final concentration for assay was 10 µg/mL.

Test surface coating

The test surface was made by transverse sectioning through a barrel of a 5-mL polypropylene syringe at 2-cm intervals. The resulting rings were then cut in half, giving rectangular surfaces of 2×1.2 cm. Polypropylene, an inert surface, was used to eliminate any contribution from the surface on the tests carried out. The surfaces were coated by placing between 10 and 20 µL of drug solution on the concave side of the surface. Surface controls included coating with diluent only and blank non-coated surfaces. All test surfaces (including blank) were allowed to dry in the BSC for 2 hours (until no solution remained). For Phase I, the surfaces were treated in the BSC and for Phase III, the surfaces were placed horizontally in the centre of the isolator, on a flat plastic tray, elevated at approximately 30 cm above ground level.

Drug recovery

Each test surface was placed into a centrifuge tube containing HPLC buffer specific for each drug (described in choice of diluent). The tubes were centrifuged for 5 minutes at $1500 \times g$. The supernatant was transferred to an autosampler vial for assay by HPLC. Recovery was determined at the experimental sample concentration. The desorption of dried drug from the polypropylene test surface into the desorbing solution (HPLC buffer) was measured against a standard (taken as 100%) which had not been subjected to these conditions (Table 1).

HPLC methods

HPLC methods,[20–22] were validated and used to quantify the amount of the parent drug remaining after all three study phases. For each assay, the flow rate was 1 mL/min, and 100 µL was injected onto the column.

5-FU: Columbus C18, 5 µm, 150×4.6 mm column and 0.01 M phosphate buffer (pH 7.0) containing 5% methanol as the mobile phase. A wavelength of 270 nm was used.

DOX: Techsphere C_{18}, 5 µm, 150×4.6 mm column and 0.01 M sodium chloride buffer (pH 2.25) containing 40% acetonitrile as the mobile phase. A wavelength of 254 nm was used.

CP: Techsphere CN, 5 µm, 250×4.6 mm column and 0.01 M ammonium sulphate buffer (pH 3.5) containing 30% methanol as the mobile phase. A wavelength of 210 nm was used.

HPLC method validation results for each assay in all three diluents are shown in Table 1.

Precision was measured as the coefficient of variation (cv) of the experimental sample concentration. Five samples were prepared and assayed on the same day (intra-day) and one sample was prepared and analysed on 5 separate days (inter-day) by HPLC. Precision was accepted if cv $> \pm 15\%$.[23]

Table 1. HPLC method validation results for 5-FU, DOX and CP in three diluents

	Cytotoxic drug		
	5-FU	DOX	CP
Limit of detection	0.2 μg/mL[a]	0.25 μg/mL[a]	2.5 μg/mL[a]
Limit of quantification	0.5 μg/mL[a]	1 μg/mL[a]	10 μg/mL[a]
Mean recovery ($n=5$)	NS = 95.9% WFI = 98.8% Buffer = 98.5%	NS = 99.9% WFI = 99.8% Buffer = 100.0%	NS = 100.4% WFI = 96.0% Buffer = 96.3%
Linearity regression coefficient	NS: $R^2 = 0.999$ WFI: $R^2 = 0.999$ Buffer: $R^2 = 0.999$	NS: $R^2 = 0.999$ WFI: $R^2 = 1$ Buffer: $R^2 = 0.999$	NS: $R^2 = 0.999$ WFI: $R^2 = 0.998$ Buffer: $R^2 = 0.997$
Intra-day precision ($n=5$)	NS: cv = 1.1% WFI: cv = 1.3% Buffer: cv = 0.7%	NS: 0.4% WFI: 1.7% Buffer: 1.9%	NS: 1.6% WFI: 1.4% Buffer: 2.2%
Inter-day precision ($n=5$)	NS: cv = 1.9% WFI: cv = 3.5% Buffer: cv = 1.5%	NS: cv = 2.5% WFI: cv = 0.4% Buffer: cv = 2.3%	NS: cv = 2.2% WFI: cv = 3.1% Buffer: cv = 3.9%

[a]*Applicable to all diluents.*

Linearity was evaluated around the expected concentration range. A correlation coefficient (R^2) > 0.99 was indicative of linearity when combined with visual inspection of the plot.

Chemical basis of the detergents (formulations)

A range of detergents (acid, neutral, alkali) was used in the study. The importance of their use is that, unlike single chemical solutions, all detergents used are composed of a series of components – such as acid/alkali/neutral base, pH regulators, oxidizing agents, chelators, surfactants and solubilizing agents – which work together to not only clean a specific target soil, such as protein from a surface, but to provide an optimum environment for the cleaning agents to work. Major components of the acidic formulations used were hydroxyacetic acid (CIP 220), phosphoric and citric acid (CIP 200), citric acid (Cage-Klenz). The neutral formulations, such as Renu-Klenz and NpH-Klenz, and the alkaline formulation, Criti-Klenz, contain high levels of surfactants, whereas the remaining alkaline formulations were potassium hydroxide, sodium hypochlorite (CIP 150) and potassium hydroxide (CIP 100) based.

Phase I: physical removal by detergents

The ability of eight detergents was investigated for their effects when used in a wipe study to physically remove dried cytotoxic drug from the test surface. WFI and IMS were used as the controls. For each detergent, 50 μL was pipetted onto a cytotoxic-coated surface and control test surface. Wiping involved one stroke across the test surface. The area was wiped immediately. If the drug remained on the surface, ie, the amount remaining was above the limit of detection of the method, a second surface of the same drug was wiped twice. If still above the limit of detection, a third surface of the same drug was wiped three times. The remaining drug was then recovered from the wiped test surface and analysed by HPLC.

Phase II: deactivation by detergents

The ability of the same eight detergents to degrade the three cytotoxic drugs was investigated using a modification of the suspension test, as set out in British Standard (BS EN 1656; 2000; 22). A 35% (Vaprox®) liquid hydrogen peroxide solution was also included to compare with the gaseous activity. A solution (100 μL) of test drug was mixed with an equal volume (100 μL) of diluted detergent or Vaprox® at room temperature. The solution was vortexed for 1 minute, then incubated at 22–23°C for up to 60 minutes. Following incubation, 800 μL of HPLC assay buffer was added to terminate the action of the detergent. IMS was used as a control. The amount of parent drug remaining in the resulting mixture was quantified by HPLC. The drugs were subjected to extreme levels of pH, ranging from 1.7 to 13.2 (Table 2).

Phase III: deactivation by VHP®

VHP® delivery and control systems have been developed to provide a consistent fumigation process for a given area.[23] VHP® was generated using 35% liquid hydrogen peroxide (Vaprox®) inside a VHP®

Table 2. Dilution and pH of detergents, Vaprox® and IMS

	Detergents									
	CIP 100	CIP 150	Criti-Klenz	Renu-Klenz	NpH-Klenz	CIP 220	Cage-Klenz	CIP 200	Vaprox®	IMS
Dilution (%)[a]	0.8	0.8	1.6	1.6	1.6	1.6	1.6	1.6	35	70
pH	13.2	12.8	11.3	8.0	7.5	2.4	2.3	1.7	2.6	5.5

[a]*All reagents used were diluted in distilled water based on manufacturers instructions, except for Vaprox®.*

100P bio-decontamination system. The VHP® 100P is a mobile system which, when connected to a given area, controls the whole dry fumigation process (Figure 1). A typical VHP® decontamination cycle consists of four phases: dehumidification, conditioning, decontamination and aeration (cycle conditions are given in Table 3). The exposure was carried out in a flexible walled isolator with an area of 1 m^3. Prior to each cycle, a leak test was carried out to ensure that the flexible isolator was leak proof. Chemical indicators were placed evenly within the isolator to confirm an even distribution of VHP®. During the course of the cycle, room and isolator temperatures were monitored for signs of condensation within the isolator. If condensation appeared, the cycle was aborted. Following exposure to VHP®, the drug was recovered from the test surfaces and quantified by HPLC.

Figure 1. Front view of a mobile VHP 100P unit, which delivers dry hydrogen peroxide vapour.

RESULTS

HPLC method validation

Validated HPLC methods for all three drugs in three diluents were used to quantify the amount of drug remaining after each test (Table 1).

Recovery from the drug-coated surfaces was very high (>95%). The methods are reproducible with a coefficient of variation of <2.2% for intra- and <3.9% inter-day precision. This applies to all three drugs in three diluents.

Linearity was demonstrated over the concentration range of 0.5–30 μg/mL for 5-FU, 10–700 μg/mL for CP, and 1–20 μg/mL for DOX. Peak area (y) plotted against concentration (x) gave a correlation coefficient (R^2) >0.99, demonstrating a linear relationship between x and y.

Phase I: physical removal by detergents

5-FU and CP were easily removed from the test surface when using a dry wipe, with acid, neutral or alkaline detergent (Table 4). DOX was easily removed with acid or neutral detergent, but was more persistent to removal by alkaline detergents, requiring more than one wipe to remove all traces (not quantifiable).

Table 3. VHP® cycle parameters

Parameter	Value
Dehumidification	
– Airflow (m^3/h)	18
– Absolute humidity (mg/L)	2.3
– Time (min)	10
Conditioning	
– Airflow (m^3/h)	12
– Injection rate (g/min)	2.5
– Time (min)	3
Decontamination	12
– Airflow (m^3/h)	1.6
– Injection rate (g/min)	25
Aeration	
– Airflow (m^3/h)	18
– Time (min)	90

Table 4. Decontamination by detergents (removal by wiping)

Test	No. of wipes required to remove drug					
	5-FU (WFI)	5-FU (NS)	CP (WFI)	CP (NS)	DOX (WFI)	DOX (NS)
WFI	1	3	1	1	1	1
Criti-Klenz	1	1	1	1	2	2
CIP 150	1	1	1	1	3	3
CIP 100	1	1	1	1	2	2
Renu-Klenz	1	1	1	1	1	1
NpH-Klenz	1	1	1	1	1	1
Cage-Klenz	1	1	1	1	1	1
CIP 220	1	1	1	1	1	1
CIP 200	1	1	1	1	1	1
IMS	1	1	1	1	1	1

An immediate colour change from red to purple was evident when the three alkaline detergents were added to DOX. The depth of purple increased with increasing alkalinity of the detergents. This colour change was not observed with the controls (WFI and IMS).

Wiping with WFI and IMS was effective in removing all three drugs. All detergents were superior over WFI when removing 5-FU diluted in NS.

Phase II: deactivation by detergents

5-FU and CP demonstrated resistance to decomposition across the pH range following 60 minutes of exposure to all detergents and liquid hydrogen peroxide. DOX was resistant to degradation at acid and neutral pH up to and including 60 minutes. However, significant degradation of DOX was observed with alkaline detergents, the rate increasing with increasing alkalinity. The rate of degradation of DOX by alkaline detergents is shown in Figure 2.

The rate of degradation of DOX can be described by the equation $y = -0.0089x + 1.9773$, $R^2 = 0.934$, and has a half-life of 31.3 minutes when exposed to Criti-Klenz.

Degradation by CIP 150 can be described by the equation $y = -0.0055x + 1.8847$, $R^2 = 0.904$, with a half-life of 33.8 minutes. Degradation by CIP 100 can be described by the equation $y = -0.009x + 1.7485$, $R^2 = 0.847$, with a half-life of 5.5 minutes.

A colour change from red to deep purple was also observed upon the addition of alkaline detergents to DOX. The depth of purple increased with increasing pH of the detergents. This was not observed with acidic- or neutral-based detergents or IMS. Temperatures, which did not exceed 24°C throughout the study, had no effect on the controls and, therefore, did not contribute to any degradation. All three drugs showed no degradation after exposure to liquid hydrogen peroxide for 60 minutes.

Phase III: deactivation by VHP®

The decontamination cycle with the VHP® was successfully completed. All chemical indicators changed colour from blue to beige during the exposure of all three drugs tested, demonstrating the presence of VHP® throughout the flexible isolator and the use of a successful validated cycle. Internal isolator temperature readings did not rise above 30°C, and external air temperature did not rise above 28°C. No condensation was predicted or evident inside the isolator at any point throughout the duration of the exposures. VHP® had no effect on the blank and control test surfaces in the study. Furthermore, there was no evidence of degradation of DOX in control test surfaces from the effect of natural light or temperature throughout the cycle duration (Table 5).

VHP® had little or no effect on 5-FU under the conditions tested. The peak area of all measurements was slightly lower than the range of accuracy of the method, but no degradation products or significant decrease in LC peak height was observed. Similarly, VHP® had no significant effect on CP. The peak area of CP diluted in buffer was slightly lower than the range of intra-day accuracy of the method, but no degradation products or decrease in peak height was observed. VHP® did cause significant degradation of DOX. The amount of degradation was dependent on the diluent used, ie, 43.4% degradation with WFI (pH 5.9), 56.0% with NS (pH 6.5), and 91.9% with buffer (pH 2.25). No colour change of DOX was observed with VHP® exposure.

Figure 3 shows a chromatogram of DOX in buffer pre-exposure (upper chromatogram) and post-exposure (lower chromatogram) to VHP®. Degradation products were not identified or quantified.

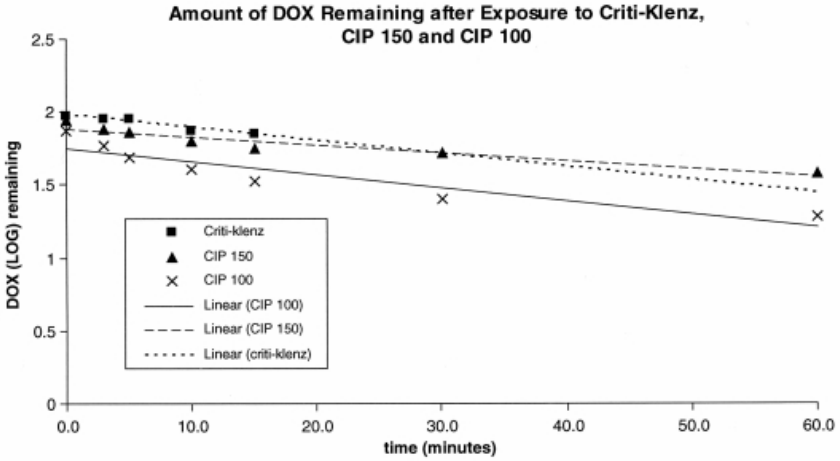

Figure 2. Amount of doxorubicin remaining after exposure to alkaline detergents.

CONCLUSION AND DISCUSSION

Many cytotoxic drugs are manipulated in clinical practice and reports of contamination by aerosols, spillages and droplets are highlighted in the literature.[3,8] This study was set up as a pilot study to investigate the effect of current decontamination technologies (VHP® fumigation and liquid detergents) as potential agents which could reduce the risk to the operator by reducing or eliminating drug contamination from a surface.

Three different cytotoxic drugs were exposed to detergents (Phase I and II) and VHP® (Phase III). Inert polypropylene test surfaces were coated with the drugs for Phases I and III. High recovery methods from the test surfaces, together with reliable HPLC methods, were developed, validated and successfully used to quantify the amount of drug remaining after testing in this study.

Table 5. VHP® exposure assay results

Drug	Exposure	Drug remaining after exposure (%)
5-FU in WFI	VHP	97.9
5-FU in NS	VHP	96.5
5-FU in buffer	VHP	99.1
CP in WFI	VHP	98.7
CP in NS	VHP	97.7
CP in buffer	VHP	94.1
DOX in WFI	VHP	56.6
DOX in WFI	Control (light)	102.4
DOX in WFI	Control (temperature)	101.0
DOX in NS	VHP	44.0
DOX in NS	Control (light)	98.9
DOX in NS	Control (temperature)	100.0
DOX in buffer	VHP	8.1
DOX in buffer	Control (light)	90.1
DOX in buffer	Control (temperature)	97.9

Preliminary wipe tests using strong alkaline, acid, or neutral detergents, and wiping with a dry wipe removed all three drugs from a contaminated surface. WFI and IMS, the controls, were also efficient. DOX was more persistent to removal, but less persistent to breakdown (indicated by a colour change) by alkaline detergents. The depth of colour change was alkaline-pH dependent.

Exposure of drug solution for up to 1 hour in strong acid and alkali and neutral-based detergents caused no degradation of 5-FU and CP. Degradation of DOX occurred when it was subjected to alkaline pH following 1 hour of incubation. This resulted in 81% degradation at pH 13.2, with 50% occurring after 5.5 minutes.

The results showed that VHP® had little to no significant effect on 5-FU or CP, however, degradation was observed with DOX. The degree of degradation was dependent on the diluent used, with significant degradation obtained when DOX was exposed following dilution in a strongly acidic buffer (pH 2.25). Further studies are in progress to investigate the effects of VHP® over longer exposure times and higher concentrations against all three drug types. It is interesting to note that liquid hydrogen peroxide, in contrast to gaseous peroxide had no effect on drug

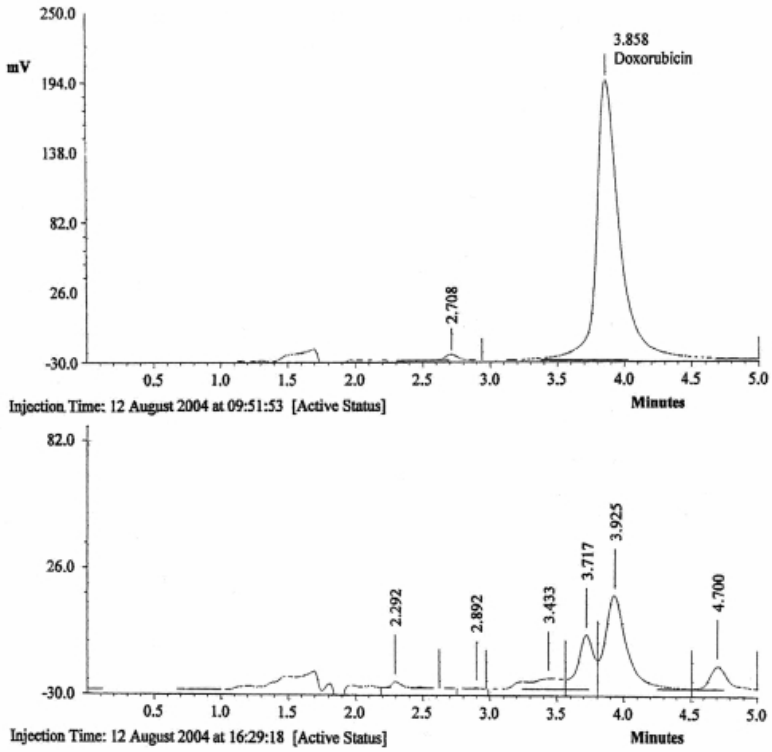

Figure 3. Example of a chromatogram of doxorubicin diluted in buffer, pre- and post-exposure to VHP®.

decomposition. The gaseous form of peroxide has been proposed to be a more effective oxidizing agent due to its unstable nature compared to liquid peroxide.[26]

This study confirms the stability of 5-FU and CP to oxidative stress, since no decomposition occurred during exposure to gaseous and liquid hydrogen peroxide, even when exposed to extreme pH conditions (pH 1.7–13.2) applied. DOX, however, was found to be less stable. The susceptibility of DOX to oxidation is reported in the literature,[24] it is also unstable at pH values <3 or >7.[25] In this study, DOX was susceptible to oxidation by VHP®, and its instability at lower pH confirmed.

Exposure of DOX to alkaline-based liquid agents resulted in a colour change from red to purple, which is indicative of decomposition. DOX was susceptible to both VHP® and alkaline agents, indicating that the degradation of DOX was occurring through two different mechanisms, oxidation as one method and alkali hydrolysis as the other method. No colour change was evident with oxidation.

The results presented are not in total agreement with the literature.[16,17] Sodium hypochlorite (5.25%) and $<30\%$ hydrogen peroxide have been reported to exhibit $>98\%$ efficiency in inactivating CP after 1 hour of exposure. DOX was also completely degraded by sodium hypochlorite. The detergent CIP 150 also contains sodium hypochlorite ($<5\%$), however, no effect was observed on CP or DOX with CIP 150, or on CP with the 35% liquid hydrogen peroxide used in this study. These results should be investigated further.

Phase I was carried out following a review of drug structures. Alkaline hydrolysis appeared to be the likely method of degradation of 5-FU in solution. A range of alkaline-based detergents was tested. Degradation of 5-FU through alkaline hydrolysis is reported to be a slow process, leading to the formation of barbituric acid, which degrades

more rapidly than it is formed, and uracil, which further degrades to urea.[27] None of the degradation products are cytotoxic. The rate of alkaline hydrolysis increases above pH 9.0.[24] Thermal and photochemical degradation causes opening of the pyrimidine ring to produce urea. To date, there is no satisfactory method for the deactivation of 5-FU.

CP degradation occurs primarily by hydrolysis in aqueous solution. The rate is constant over the pH range 2–10. Specific acid and specific base catalysis occurs at extreme pH.[27] Under acidic conditions, hydrolysis may occur by different pathways. The pathway and breakdown products formed depend upon the pH of the solution.[27] Under basic or neutral conditions, hydrolysis occurs by an initial intramolecular alkylation, forming a bicyclic compound and hydrochloric acid.[27] However, this compound is very labile in aqueous solution and may breakdown to further products.[27] CP is temperature sensitive and hydrolysis may occur rapidly at temperatures above 30°C.

DOX exhibits pH-dependent stability in solution. It is sensitive to light and temperature. In solutions of pH <4, the glycosidic bond is cleaved, releasing a red water-soluble amino sugar (daunosamine),[25] and a water-insoluble tetracyclic aglycone (doxorubicinone). The aglycone is cytotoxic, but less so than the parent drug.[24]

In alkaline solution, a colour change from red to deep purple is due to rapid degradation of the drug.[24] It is thought to reflect cleavage of the amino sugar and the formation of other degradation products, such as 7,8-dehydro-9,10 desacetyl-daunorubicinone.[24] This colour change also occurs with the other anthracyclines antibiotics which are structurally similar.[24,25]

The results of this study demonstrate that current decontamination methods can be used to reduce, if not eliminate, the risk posed by cytotoxic drugs to the operator and environment. The extent of risk reduction or elimination will vary depending on the structure of the drug. Oxidation and alkaline-based detergents have resulted in the breakdown of DOX by two different mechanisms. Oxidation and alkaline hydrolysis may be used to investigate their effects on drugs of the same anthracycline family as DOX. However, limited success was observed with the chemical deactivation technologies used in this study when applied to drugs representing the other classes, ie, the alkylating agents and the anti-metabolites. Further studies are required to investigate these classes of drugs. The liquid detergents used in this study were formulated for specific target soil. It may be necessary to vary the formulation to target the more stable structures of 5-FU or CP.

The solubility of a drug depends upon the extent to which it is ionized. This is determined by the pKa of any acidic and basic groups and the pH of the environment. The drugs are more soluble and likely to be taken up by a wipe at the pH at which they are 100% ionized. DOX is ionized at low pH and was easier to remove from the surface with acid rather than alkali detergents, however alkaline detergents played a part in DOX degradation.

All three drugs used in this study are polar molecules (log $P<1$) and were removed from a coated surface by water and aqueous-based detergents. Most cytotoxic drugs are water-soluble, therefore, it is recommended that decontamination is removal by the use of wipes impregnated with an aqueous-based agent, which binds the target drug, followed by disposal of the wipe. A combination of agents will be required to cover removal, ie, it is possible that a high pH may degrade the drug to compounds that are more soluble and are easier to remove at low pH. Wiping with IMS is common practice, but may also play a part in the removal of less water-soluble drugs and drug degradation products.

From this study, it is suggested that a review of cleaning practices should be carried out and a protocol developed based on the recommendation that after cleaning with water, surfaces should be cleaned with detergent of high pH, followed by detergent of low pH, finally wiping with IMS. VHP is an established process for biological control in isolators and may also be considered, together with other measures, for the control of contamination by drugs that are readily oxidized, such as the anthracyclines.

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Safe handling of cytotoxics

Despite the adoption of standard protective measures, healthcare workers are still exposed to cytotoxic drugs. The risks to the operator and the environment may be reduced by considering effective decontamination and intervention methods

Sarah Roberts
BSc

PhD Student

Department of Pharmacy and Pharmacology
University of Bath
Bath
UK

Graham Sewell
PhD

Professor

Department of Pharmacy
Kingston University
Kingston-upon-Thames
UK

E: s.roberts@kingston.ac.uk

To protect the worker when handling cytotoxic drugs, more is needed than careful use of personal protective equipment and techniques. Despite the implementation of collective protective measures, many international studies have demonstrated levels of cytotoxic contamination in the surrounding environment and in the urine of operators.[1-6] The risk posed by cytotoxic drugs to the operator and the environment may be reduced, if not eliminated, by considering additional approaches: firstly, the application of effective decontamination methods, and secondly, the use of intervention (ie, a closed-system drug transfer device).

Routes of exposure

Cytotoxic contamination in the workplace and subsequent worker exposure may arise from several sources, even in the absence of manipulation errors. Figure 1 shows leakage onto the base of the isolator chamber which occurred when quinine sulphate was used as a fluorescent marker simulating cytotoxic manipulation in an isolator. The event occurred when withdrawing a syringe needle after addition to an infusion bag. Such surface contamination could be transferred to any surface coming into direct contact with it (eg, contact with gloves could later result in dermal exposure through glove penetration, or in transfer of contamination to further surfaces).[7] Successive use of the same syringe has also been implicated as a source of glove contamination by cyclophosphamide and of potential dermal contact with cyclophosphamide.[8]

If such surface contamination were not immediately removed, it could dry out, become airborne and contribute towards inhalation. It has been suggested that dried drug particles on surfaces or on the high-efficiency particulate air (HEPA) filter of the isolator or biological safety cabinet (BSC) may act as a reservoir, providing a source area from which molecules could slowly vaporise.[9] Some drugs, such as cyclophosphamide, can vaporise at room temperature or higher – and certainly, temperatures higher than room temperature may be reached under normal conditions in which cytotoxic drugs are manipulated, with factors such as continual running of isolators and BSCs, and ongoing use of ancillary equipment such as lighting and pump motors.[10]

Production of aerosols has also been demonstrated as a source of contamination when applying techniques used to reconstitute and dilute cytotoxic drugs.[11,12] Oral ingestion or accidental dermal exposure from needlestick injuries, spillages or breakages could also occur.[13] These events happen relatively less often, but can contribute to airborne and long-term workplace contamination.

Precontaminated sources taken into the environment before any manipulations are carried out will contaminate gloves and any surfaces subsequently touched. It has been demonstrated that the external surfaces of drug vials may be contaminated with the drug contained, which could also contaminate packaging. Levels found varied according to origin, indicating that precautionary measures taken are not standardised.[14-17] Contamination on vials' external surfaces may occur due to splashing, foaming or dusting during the filling process. This may be reduced by vial washing and subsequent application of protective sleeves.[14]

In the UK and France, current practice in hospital pharmacies is to use negative-pressure isolators.[18] Isolators offer containment, but there is no evidence for their superiority over BSCs. Isolators have their limitations and there is the potential for contamination to pass through hatches from the main chamber to the environment. Cytotoxic contamination has been reported on interior surfaces and on surfaces of the finished product in isolators and BSCs.[19]

If vaporisation were a common occurrence, the HEPA filters of both isolators and BSCs would not be effective in retaining molecules of cytotoxic vapour smaller than the filter's pore size. As a result, any cytotoxic aerosols generated would pass through the filter and be released into the environment. Therefore, whichever source is used, it is paramount to operator protection that the air be exhausted externally, away from the working environment.[13]

Guidelines

Recommendations on good practice have been produced by the Occupational Health and Safety

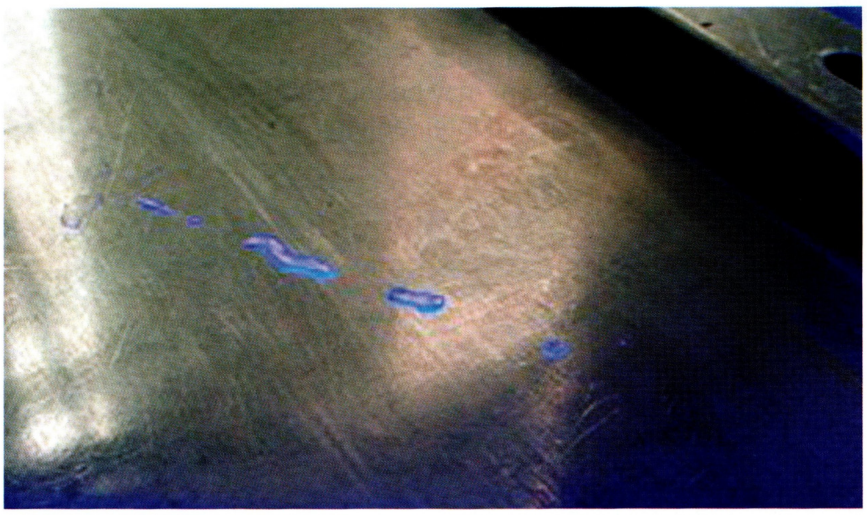

Figure 1. Contamination from a solution of quinine sulphate produced when withdrawing a syringe after addition to an infusion bag

Administration and the American Society of Health-System Pharmacists in the USA; by the International Society of Oncology Pharmacy Practitioners; by the Health and Safety Executive and the Royal College of Nursing, and the Management and Awareness of the Risks of Cytotoxic Handling group in the UK.[20-24]

Decontamination methods

EPA

Agents chosen for decontamination in areas where cytotoxic drugs are compounded are mainly intended for biological decontamination. Decontamination protocols should be carefully designed and validated to confirm a biological "kill," and likewise to confirm the removal or chemical degradation of cytotoxic drugs. Different biological agents have varying modes of action and efficacies against different micro-organisms; the same applies to cytotoxic drugs, which represent a diverse range of chemical structures.

It is recommended that all surfaces be cleaned according to a protocol, which includes an appropriate deactivation agent to facilitate the removal and/or breakdown of biological and chemical contamination.[13] Water-soluble cytotoxic drugs should be removed using wipes impregnated with an aqueous-based agent which binds the target drug, and the wipe should be disposed of afterwards.[25] The drug would be more likely to be picked up by applying a detergent formulated at a pH at which the target drug is ionised. One should consider the possibility that surface contamination may take the form of multidrug chemical contamination, since cytotoxic drugs are frequently handled simultaneously; here, a combination of agents may be required. The chemistry of any breakdown products (which may retain cytotoxic activity) should also be considered alongside removal of the target drug. Wiping with industrial methylated spirit is common practice, but may also play a part in the removal of less water-soluble drugs and degradation products.[25]

Intervention with closed-system containment device

The US National Institute for Occupational Safety and Health recommends the use of a closed-system drug transfer device to prepare cytotoxic drugs, and this has been acknowledged by the relevant European directive and by international guidelines.[13,23,26-28]

Studies carried out at research centres worldwide, using the PhaSeal® closed-system drug transfer device have all demonstrated a significant reduction of contamination.[29-33]

Preventing contamination at source is more effective than trying to remove the contamination once it has occurred. Eliminating the primary contamination event will also prevent secondary contamination of areas outside the immediate drug environment.[30]

However, while closed-system devices may have a role in minimising staff exposure, one cannot recommend that they replace trained pharmacy staff using personal protective equipment and a pharmaceutical isolator.[34] Containment by the device

Resources

US Occupational Health and Safety Administration
W: www.osha.gov

American Society of Health-System Pharmacists
W: www.ashp.org

International Society of Oncology Pharmacy Practitioners
W: www.isopp.org

UK Health and Safety Executive
W: www hse.gov.uk

UK Royal College of Nursing
W: www.rcn.org.uk

Management and Awareness of the Risks of Cytotoxic Handling (MARCH)
W: www.marchguidelines.com

can only be achieved in the area in which the device is used – there would be no influence over pre-contaminated sources brought into the pharmacy.

Conclusions

Despite the adoption of standard protective measures, healthcare workers continue to be exposed to cytotoxic drugs. Targeting decontamination to the chemistry of the drugs used should be considered, as should the implementation of a closed-system device. Manufacturers should also be encouraged to improve their decontamination procedures and to guarantee the supply of vials free of cytotoxic contamination. ■

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ISOPP Abstract

Studies on the Decontamination of Cytotoxic Drugs on Surfaces in Chemotherapy Workstations.

S. Roberts, N.Khammo, G.McDonnell, M.Bernado, G.J.Sewell.

Objectives: Evaluate the effect of decontamination methods currently used in the pharmaceutical industry to degrade 3 cytotoxic drugs.

Methods: Study was completed in three phases: Phase I investigated the ability of three alkaline detergents to remove physically cytotoxic drugs from a surface (this could be incorporated into a cleaning protocol); Phase II investigated the ability of eight water-soluble detergents (acidic, alkaline and neutral), and liquid hydrogen peroxide to degrade cytotoxic drugs; and Phase III investigated the ability of 35% Vaporized Hydrogen Peroxide (VHP®) to degrade cytotoxic contamination on an inert surface by oxidation. VHP® technology is based on a dry process and drug exposure was carried out at a rate of 1.6g/min for 25 min. The amount of drug after each phase was quantified by high recovery HPLC methods. 5-Flurouracil, doxorubicin and cyclophosphamide were removed by wiping the surface with alkaline detergents.

Results: Degradation of doxorubicin was observed after 1 hour of exposure to alkaline detergents, with 81% observed at pH 13.2. Doxorubicin changed colour from red to purple under alkaline conditions, which is indicative of degradation. Doxorubicin was chemically stable in neutral and acid detergents. VHP® and alkaline detergents caused degradation of doxorubicin. The effect was diluent and pH dependent; 43.4% degradation occurred in water for injections (pH 5.9), 56.0% in 0.9% sodium chloride (pH 6.5), and 91.9% in buffer (0.01 M, pH 2.25).

Conclusion: 5-Flurouracil and cyclophosphamide were not susceptible to chemical degradation by both technologies, at the concentrations and contact times tested. Alternative methods need to be explored.

Studies on the Decontamination of Cytotoxic Drugs on Surfaces in Chemotherapy Workstations

Sarah Roberts[1], Nancy Khammo[2], Gerald McDonnell[2], Mildred Bernado[3] and Graham Sewell[4]

[1]Department of Pharmacy and Pharmacology, University of Bath, Bath. UK. [2]Steris Ltd, Basingstoke. UK. [3]Steris Corp, USA. [4]Department of Pharmacy, Kingston University. London. UK.

Contact: prssr@bath.ac.uk

Poster number 22

Introduction

During the preparation of cytotoxic infusions, a variety of drug manipulations are performed resulting in the production of aerosols and droplets, which are known to contaminate the areas which they disperse into including isolators and surrounding surfaces (1-6). This increases the risk of occupational exposure to these drugs. The health effects resulting from their exposures are well documented (7-10).

Chemical and biological decontamination are both important in the safe dispensing of cytotoxic drugs. However, the effectiveness of removing cytotoxic residues from surfaces during cleaning is not often considered. Ideally, removing contamination should involve the physical removal of drug from a surface and breakdown into less cytotoxic compounds.

Aims

The aims of this study were to investigate the safe decontamination of cytotoxic drugs by removal and breakdown into safe by-products. This was investigated by two decontamination technologies currently used in various hospital and pharmaceutical applications.

The study was carried out in three phases on three marker cytotoxic drugs; 5-fluorouracil (5-FU; an antimetabolite), cyclophosphamide (CP; an alkylating agent) and doxorubicin (DOX; an anthracycline).

Methods

Figure 1. Front view of a mobile VHP® 100P Biodecontamination unit.

Eight liquid detergents (pH 1.7 – 13.2), diluted as per manufacturers instructions were investigated as cleaning agents (phase I and II);

Phase I

Detergent was pipetted onto a contaminated surface. The surface was wiped and the ease of removal evaluated.

Phase II

Equal volumes of drug + detergent were mixed, incubated together, and the reaction terminated at time intervals up to and including 1 hour.

Determination of Vaporised Hydrogen Peroxide (VHP®) to oxidise cytotoxic contamination was investigated (phase III);

Phase III

Drugs were exposed to VHP in a flexible walled isolator. VHP was generated from a VHP® 100P biodecontamination system (Figure 1) that, when connected to a given area, controls the whole dry fumigation process. The VHP is a dry gas that is generated in the system from a liquid peroxide solution (Vaprox®). The full VHP cycles consists of 4 phases: dehumidification, conditioning, decontamination and aeration (11). Exposure to VHP in test cycles was for 25 minutes.

Recovery coupled with HPLC methods were developed and validated to quantify the amount of drug remaining on the surface (phase I and III), and degradation of the parent drug (phase II).

Results

Phase I

5-FU and CP were easily removed from the test surface when wiped using a dry wipe and acid, neutral or alkaline detergents.

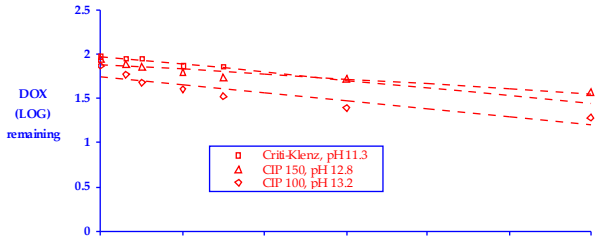
DOX was easily removed with acid and neutral detergent but was more resistant to removal by alkaline detergents.

Phase II

5-FU and CP demonstrated resistance to degradation across the pH range following 1 hour of exposure to all detergents.

DOX was resistant to degradation at acidic and neutral pH following 1 hour of exposure. Significant degradation of DOX occurred when it was exposed to alkaline detergents (Criti-klenz, CIP 150 and CIP 100). This resulted in 81% degradation at pH 13.2, with 50% occurring after 5.5 minutes (Figure 2).

Figure 2. Degradation of DOX by Alkaline Detergents.



In both phases I and II a colour change of DOX from red to purple was observed. The depth of purple increased with increasing alkalinity. This is indicative of alkaline degradation of DOX.

Phase III

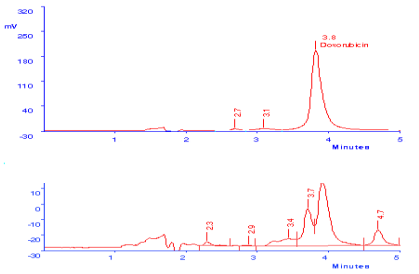
VHP had no effect on 5-FU or CP, but caused degradation of DOX. The amount of degradation was dependent on the diluent used (Table 1).

Table 1. Percentage Degradation of DOX by VHP.

Diluent	pH of Diluent	Percentage Degradation
0.9% Sodium Chloride	6.5	56.0
Water for Injections	5.9	43.3
Buffer	2.25	91.9

Significant degradation (91.9%) was obtained when DOX was exposed diluted in a strongly acidic buffer (Figure 3).

Figure 3. Chromatogram of DOX diluted in buffer, pre (upper) and post (lower) exposure to VHP.



Conclusions and Discussion

Strong acid, alkaline, or neutral detergents removed all three drugs from a contaminated surface. DOX was more resistant to removal by alkaline detergents, but less resistant to breakdown.

The stability of 5-FU and CP to oxidative stress was demonstrated, since no degradation occurred following exposure to VHP. In addition, no degradation occurred under the extreme pH conditions (1.7 - 13.2) applied.

DOX was, however, found to be less stable, with 81% degradation occurring after 1 hour of exposure to the alkaline detergent, CIP 100. Oxidation, aided by acid hydrolysis also caused significant degradation of DOX, as observed when exposed to VHP; interestingly, unlike the VHP results, degradation was not observed with liquid/condensed hydrogen peroxide at 30%v/v (results not shown).

This study has demonstrated the breakdown of DOX (an anthracycline antibiotic) by two different mechanisms (oxidation and alkaline hydrolysis), however limited success was observed on drugs representing other classes i.e. 5-FU and CP. The results of this study demonstrate that current decontamination methods can be used to reduce, if not eliminate the risk posed by cytotoxic drugs to the operator and environment. The liquid detergents used in this study were formulated for specific target soil. Future studies may include varying the formulation of the detergent to target the more stable structures of 5-FU or CP. Alternatively, removal by the use of a wipe incorporating an agent which binds the target drug could be a compromise. DOX was susceptible to VHP; further studies with VHP may include longer exposure times with DOX and other cytotoxic drugs.

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April 5th, 2006

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Appendix 1 – Research Ethics Committee Approval

South West Devon Research Ethics Committee
Room 101B
ITTC South Building
Tamar Science Park
Davy Road
Derriford
PLYMOUTH
PL6 8BX

Telephone: 01752 315267
Facsimile: 01752 315268
Email: halina.pounds@phnt.swest.nhs.uk

11 October 2006

Miss Sarah Roberts
Research Student
University of Bath
Pharmacy, University of Bath,
Claverton Down, Bath
BA2 7AY

Dear Miss Roberts

Full title of study: **Investigation into the Decontamination (cleaning and disinfection)Procedures Carried out after the Manipulation of Cytotoxic Drugs: A Questionnaire Survey of Current Practice in the UK.**

REC reference number: **06/Q2103/120**

The Research Ethics Committee reviewed the above application at the meeting held on 10 October 2006.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation.

However the Committee suggested that you remove the first two questions ie name and job title from the questionnaire to assist in maintaining confidentiality.

Ethical review of research sites

The Committee agreed that all sites in this study should be exempt from site-specific assessment (SSA). There is no need to complete Part C of the application form or to inform Local Research Ethics Committees (LRECs) about the research. The favourable opinion for the study applies to all sites involved in the research.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Application		12 September 2006
Investigator CV	1	12 September 2006
Protocol	1	12 September 2006
Letter from Sponsor		11 September 2006
Peer Review		12 September 2006
Questionnaire	1	12 September 2006
Letter of invitation to participant	1	12 September 2006
CV for Graham Sewell	1	12 September 2006
Evidence of Insurance		13 September 2006

Research governance approval

You should arrange for the R&D Department at all relevant NHS care organisations to be notified that the research will be taking place, and provide a copy of the REC application, the protocol and this letter.

All researchers and research collaborators who will be participating in the research at a NHS site must obtain final research governance approval before commencing any research procedures. Where a substantive contract is not held with the care organisation, it may be necessary for an honorary contract to be issued before approval for the research can be given.

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

06/Q2103/120	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

MR A. J. R BEAUCHAMP
Dip. Healthcare Ethics
Chairman

Appendix 2 – Questionnaire to Investigate Decontamination Procedures Carried out after the Manipulation of Cytotoxic Drugs

The purpose of this questionnaire is to learn about the current decontamination (cleaning and disinfection) procedures which take place in hospitals in the U.K. where cytotoxic drugs are compounded.

This questionnaire should take approximately 10-15 mins to complete.

Please click and type in the boxes below

Hospital

Question 1.

In your hospital what type of workstation do you use for the compounding of cytotoxic drugs?

Please click in the box either yes or no

	Yes	No
a. in an internally-ducted positive-pressure isolator	☐	☐
b. in an externally-ducted positive-pressure isolator	☐	☐
c. in an internally-ducted negative-pressure isolator	☐	☐
d. in an externally-ducted negative-pressure isolator	☐	☐
e. in an internally-ducted biological safety cabinet	☐	☐
f. in an externally-ducted biological safety cabinet	☐	☐

g. other, ***please specify by clicking in the top left corner and typing in the box below***

The following questions 2-8 concern the main area answered in question 1

Question 2.
How would you describe your concerns about cytotoxic contamination in this area? ***Please click in the appropriate box***

a. extremely concerned ☐

b. slightly concerned ☐

c. indifferent ☐

d. not concerned at all ☐

Question 3.
Do you apply a 'cleaning' and a 'disinfection' procedure to this area?
Please click in the appropriate box

a. a cleaning procedure only ☐ **go to question 4**

b. a disinfection procedure only ☐ **go straight to question 5**

c. a cleaning and a disinfection procedure ☐ **go to question 4**

Question 4.
Which of the following do you use for the <u>cleaning</u> of this area?
Please specify frequency of use <u>either</u> daily, weekly or monthly
(click and select from the drop down menu in the appropriate box)

	daily	**weekly**	**monthly**
a. water			
water for injections	0	0	0
water for irrigation	0	0	0
distilled water	0	0	0
deionised water	0	0	0
other water quality	0	0	0
b. 70/30 alcohol			
IMS spray	0	0	0
IPA spray	0	0	0
IMS impregnated wipes	0	0	0
IPA impregnated wipes	0	0	0
other alcohol	0	0	0

c. detergents, please list product and manufacturer
please list product and manufacturer by clicking and typing in the box below

daily	weekly	monthly
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0

Question 5.
Which of the following do you use for the disinfection of this area?
Please specify application and frequency of use either daily, weekly or monthly?
(click and select from the drop down menu in the appropriate box)

Part 1. Liquid Biocides	**application**	**daily**	**weekly**	**monthly**	**yearly**
a. 70/30 alcohol	none	0	0	0	0
b. stabilised chlorine dioxide	none	0	0	0	0
c. quaternary ammonium	none	0	0	0	0
d. quaternary ammonium/stabilised chlorine dioxide	none	0	0	0	0
e. sodium hypochlorite	none	0	0	0	0
f. hydrogen peroxide	none	0	0	0	0
g. stabilised glutaraldehyde	none	0	0	0	0
h. chlorhexidine	none	0	0	0	0
i. other	none	0	0	0	0

please specify by clicking in the top left corner and typing in the box below

Part 2. Gassing/Fumigation	**daily**	**weekly**	**monthly**	**yearly**
a. formaldehyde	0	0	0	0
b. hydrogen peroxide	0	0	0	0

if applicable please give details of the system by clicking and typing in the box below

c. peracetic acid vapour	0	0	0	0
d. ozone gas	0	0	0	0
e. other	0	0	0	0

please specify by clicking in the top left corner and typing in the box below

Question 6.
Does the decontamination procedure for this area differ depending upon which drugs have been manipulated?
Please click either yes or no

Yes ☐ No ☐

If yes please give details by clicking in the top left corner and typing in the box below

Question 7.
In your opinion which <u>one</u> of the following <u>best</u> describes the purpose of the decontamination procedure which you have described?
Please click in one of the boxes below

a. to remove cytotoxic contamination ☐

b. to disinfect/sterilise the area ☐

c. to maintain a sterile environment and remove some cytotoxic contamination ☐

d. to maintain a sterile environment and remove all cytotoxic contamination ☐

Question 8.
How was the decontamination procedure you use devised?
Please click either yes or no
If yes please give details by clicking in the top left corner and typing in the box below

a. from national guidelines **Yes** ☐ **No** ☐

b. from international guidelines **Yes** ☐ **No** ☐

c. literature/references **Yes** ☐ **No** ☐

d. other facilities **Yes** ☐ **No** ☐

e. manufacturers advice **Yes** ☐ **No** ☐

f. other***, please specify*** **Yes** ☐ **No** ☐

Question 9.
Have you ever measured for cytotoxic contamination in the following areas, and if so was any contamination found?
Please click either yes or no

	Yes	**No**		**Yes**	**No**
a. vials	☐	☐	contamination found?	☐	☐
b. the dispensed product	☐	☐	contamination found?	☐	☐
c. surface inside the BSC/isolator					
i. before decontamination	☐	☐	contamination found?	☐	☐
ii. after decontamination	☐	☐	contamination found?	☐	☐
d. surface outside the BSC/isolator					
i. before decontamination	☐	☐	contamination found?	☐	☐
ii. after decontamination	☐	☐	contamination found?	☐	☐
e. airborne	☐	☐	contamination found?	☐	☐
f. gloves used in the BSC/isolator	☐	☐	contamination found?	☐	☐
g. gloves used outside the BSC /isolator	☐	☐	contamination found?	☐	☐
h. other areas	☐	☐	contamination found?	☐	☐

if yes, please give details by clicking in the top left hand corner and typing in the box below

Question 10.
Please comment on any concerns you have about manipulating cytotoxic drugs and any problems which you have experienced.
Please click in the top left corner and type in the box below

Appendix 3 – Letter of Invitation

Letter of Invitation

Dear Sir/Madam,

I am a research student at the University of Bath and as part of my PhD I am researching the current practice of decontamination procedures carried out in hospital pharmacies where cytotoxic drugs are compounded.

Please find enclosed a research questionnaire titled ***"Questionnaire to Investigate Decontamination Procedures Carried out after the Manipulation of Cytotoxic Drugs".*** I have sent this questionnaire to you because information from your hospital pharmacy would make a valuable contribution to this research.
(If this is not applicable to you please would you pass it on to the appropriate person).

The questionnaire is not compulsory and I appreciate that you are busy, but it would be very much appreciated if you would take the time to complete it. If you would like to take part in this research click on the questionnaire to open it and

EITHER
1. save it to the desktop, fill it out by following the instructions and email back it to me at s.roberts@bath.ac.uk OR
2. print off a copy, fill it out by hand and return to me at the freepost address below

Sarah Roberts
Department of Pharmacy and Pharmacology
FREEPOST (SN1548)
Bath. BA2 7LZ

The information will remain anonymous and will be used for research purposes only.

Any questions or queries please do not hesitate to contact me
s.roberts@bath.ac.uk
Tel: 01225 384423

Many thanks

Sarah

Research Student
Pharmacy
University of Bath

Appendix 4 – Chromatograms

Figure 55. Example of a Chromatogram of Cyclophosphamide Recovered from a Wipe

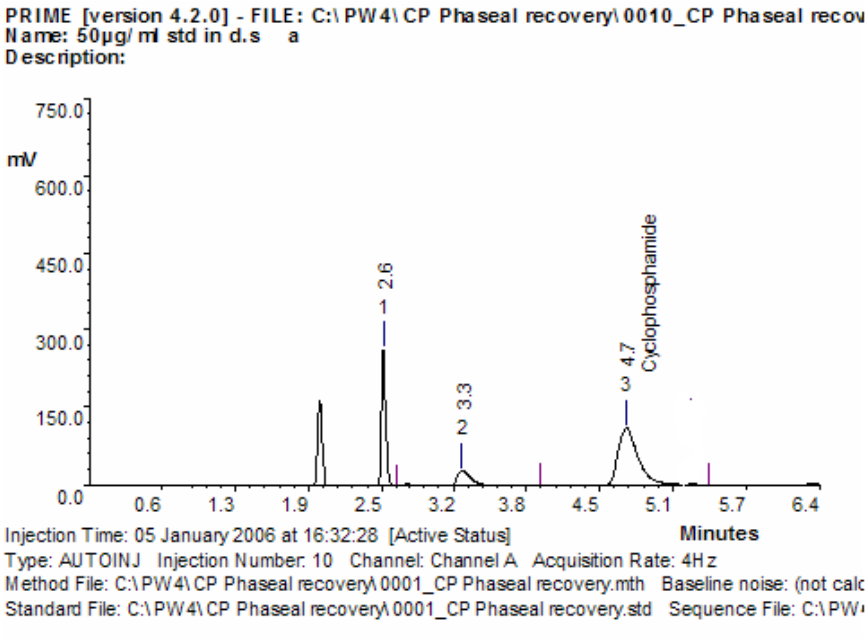


Figure 56. Example of a Chromatogram of EPI Recovered from an Isolator Glove

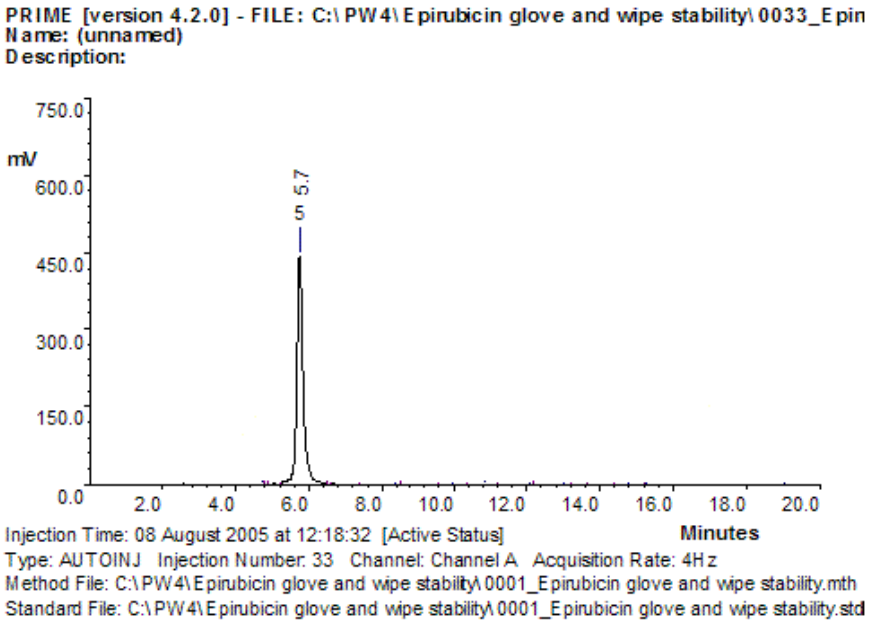


Figure 57. Example of a Chromatogram of MTX Recovered from a Wipe

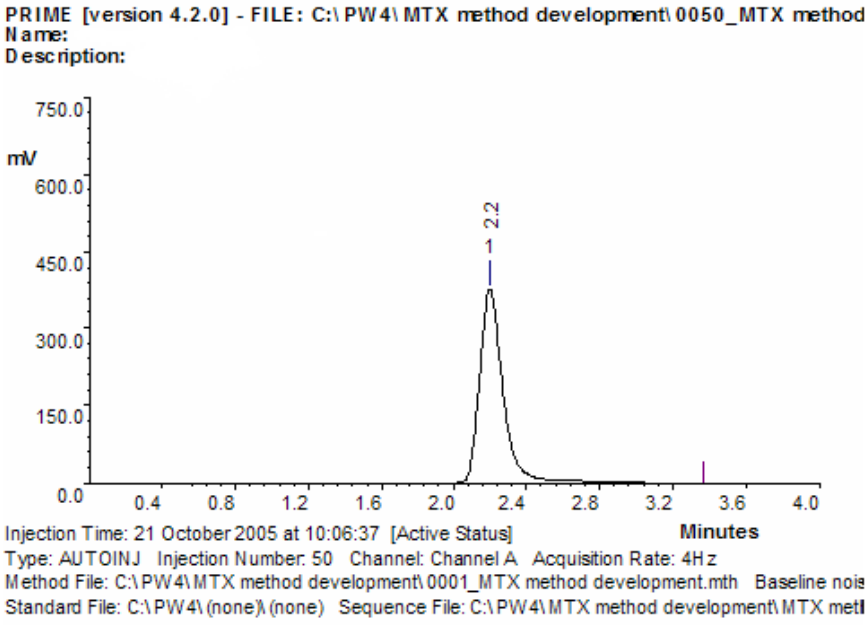


Figure 58. Example of a Chromatogram of DOX (buffer) Standard (no exposure to VHP®)

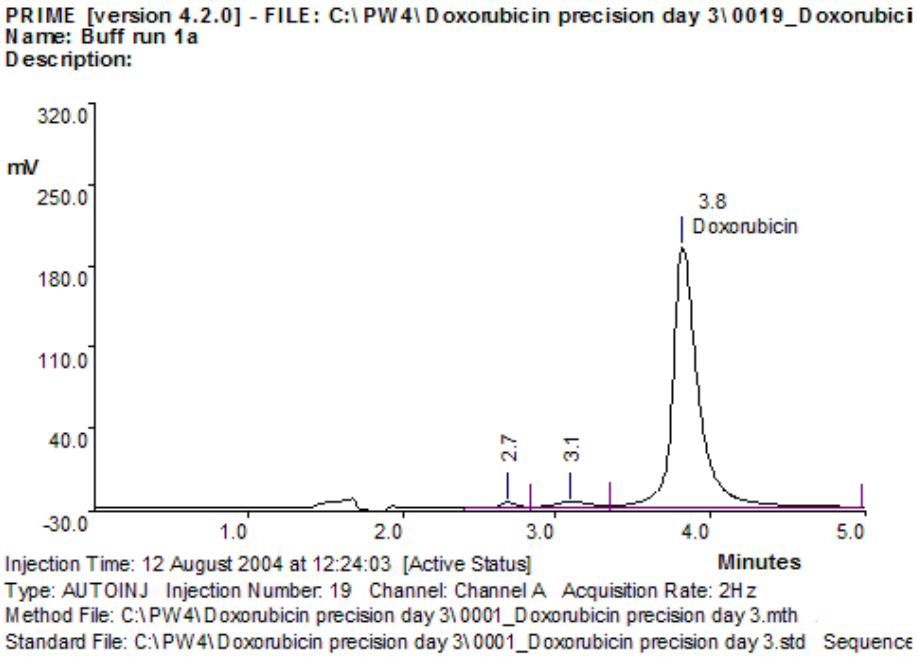
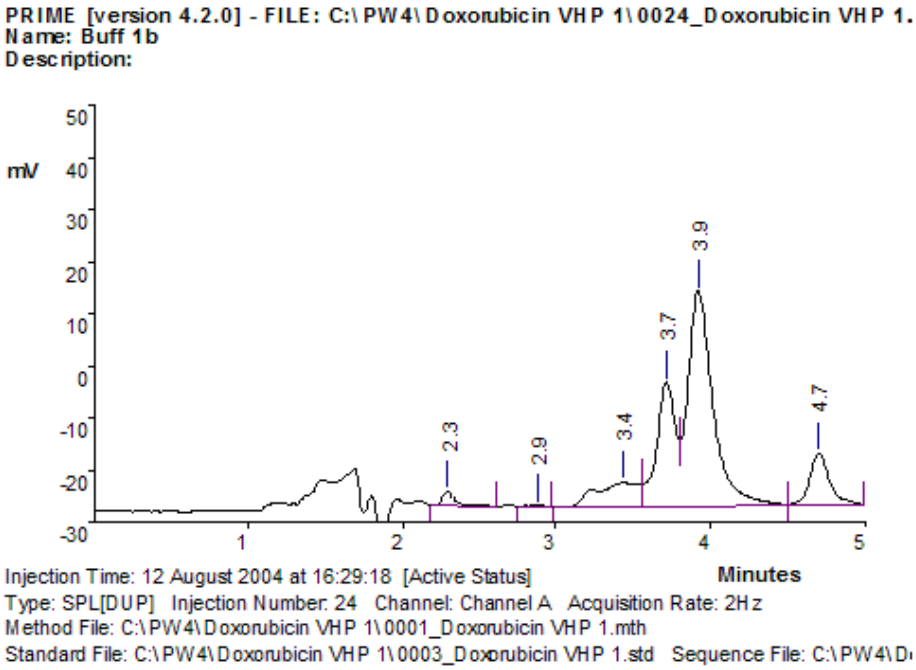


Figure 59. Example of a Chromatogram of DOX (buffer) Post Exposure to VHP® Cycle 1



Appendix 5 – List of Standard Operating Procedures

Pharmacy Technical Services Department
Plymouth Hospitals NHS Trust, Derriford

SOP CG6 (Version 2) Changing Procedure- To Enter Isolator Rooms

SOP ASG2 (Version 3) Aseptic Suite Transfer Hatch Procedure

SOP ASG8 (Version 1) Reconstitution Procedure

SOP ASG17 (Version 3) Assembly and Manufacture of Syringes Produced Under the Specials Manufacturing License

SOP ASG30 (Version 2) Tray Cleaning Procedure

SOP CH1 (Version 3) Procedure for the Preparation of Cytotoxic Injectables Under Section 10 Exemption of Medicines Act

SOP CH7 (Version 3) Cytotoxic Envair Isolator Procedure for Use

SOP CH8 (Version 3) Envair Cytotoxic Isolator Glove and Sleeve Changing Procedure

SOP CH9 (Version 2) Procedure for Dealing with Cytotoxic Spillages

Appendix 6 – Questionnaire (closed-system device)

Questionnaire to Determine Operators' Opinion on the Use of the Closed-System (PhaSeal®) Device

Name of Operator ……..**Date:…….**

Part 1. To be completed by the operator:
Q1.a. How long have you been working with cytotoxic drugs? Describe experience

…….

…….

…….

Q1.b. How worried are you about working with cytotoxic drugs? Please circle

- Very worried
- Worried
- Slight bothered
- Not bothered at all
- Quite happy

Q1.c. How safe do you feel with the current methods you have been using when working with cytotoxic drugs? Please circle

- Very safe
- Safe
- Neither safe nor unsafe
- Unsafe
- Very unsafe

Comments……...

…….

…….

…….

Part 2. To be completed by the operator at the start of intervention period (1):

Q2.a. I found the training for the closed-system device adequate. Please circle

- Strongly agree
- Agree
- Neither agree or disagree
- Disagree
- Strongly disagree

Q2.b. I feel confident using the closed-system device after the training given. Please circle

- Strongly agree
- Agree
- Neither agree or disagree
- Disagree
- Strongly disagree

Q2.c. Do you have any reservations about using the closed-system device? If so please describe

…….

…….

…….

Q2.d. How confident are you that the closed-system device is protecting you? Please circle

- Very confident
- Confident
- Neither confident nor unconfident
- No confidence
- Not confident at all

Signature of operator:……. Date:……...

Part 3. To be completed by the operator at the end of intervention period (1):

Q3.a. How confident are you in using the closed-system device now? Please circle

- Very confident
- Confident
- Neither confident nor unconfident
- No confidence
- Not confident at all

Q3.b. I was more careful when using the closed-system device than when using the open-system. Please circle

- Strongly agree
- Agree
- Neither agree nor disagree
- Disagree
- Strongly disagree

Q3.c. Did you have any problems when using the closed-system device? If so please describe

…….

…….

…….

…….

Q3.d. Did using the closed-system device hinder work in any way? Please describe

…….

…….

…….

…….

Q3.f. After using the closed-system device which method would you prefer to continue working with? Please circle

- Open-system
- Closed-system

and explain why

…….

…….

…….

…….

…….

Signature of operator:……. Date:…….

Appendix 7 – Epirubicin Surface Contamination Data

Table 59. Amount of EPI Recovered from Isolator Surfaces during Baseline 1 and 2

Area	Day and Sampling Point															
	1a	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**
Baseline 1 (ng cm^{-2}, or ng per sleeve)																
Base	0.03	0.59	0.17	0.11	0.20	<0.002	0.09	0.08	0.08	0.07	0.05	<0.002	0.05	0.04	0.07	0.06
Door, outer (R)	ND	0.07	0.07	0.05	0.06	<0.003	ND	ND	ND	ND	ND	ND	ND	ND	0.07	ND
Door, outer (L)	ND	0.01	<0.003	0.05	<0.003	ND	<0.003	ND	ND	<0.003	ND	ND	ND	ND	0.07	ND
Door, inner (R)	<0.003	0.08	<0.003	0.07	<0.003	0.06	ND	ND	<0.003	ND	ND	ND	ND	ND	0.07	ND
Door, inner (L)	0.003	0.08	ND	0.07	0.05	0.07	ND	ND	ND	<0.003	<0.003	ND	ND	<0.003	<0.003	ND
Sleeve (R)	<2.0	711	89.5	90.5	106	41.0	41.5	ND	41.5	42.5	45.0	ND	ND	ND	49.0	ND
Sleeve (L)	ND	398	86.0	81.0	44.0	<2.0	<2.0	ND	<2.0	<2.0	ND	ND	<2.0	<2.0	49.5	53.5
Baseline 2 (ng cm^{-2}, or ng per sleeve)																
Base	ND	ND	ND	ND	ND	<0.002	<0.002	ND	<0.002	ND	ND	ND	ND	ND	ND	ND
Door, outer (R)	ND	ND	ND	ND	ND	ND	<0.003	ND	ND	ND	ND	<0.003	ND	ND	ND	ND
Door, outer (L)	ND	ND	ND	ND	<0.003	ND	<0.003	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, inner (R)	ND	ND	ND	ND	<0.003	<0.003	ND	ND	ND	ND	ND	0.05	ND	ND	ND	ND
Door, inner (L)	ND	ND	ND	ND	<0.003	<0.003	<0.003	ND	ND	ND	<0.003	ND	ND	ND	ND	<0.003
Sleeve (R)	ND	ND	ND	ND	ND	ND	<2.0	<2.0	ND	ND	ND	<2.0	ND	ND	ND	ND
Sleeve (L)	ND	ND	ND	ND	ND	ND	<2.0	<2.0	ND	ND	ND	ND	ND	<2.0	ND	ND

EPI LoD (ng cm^{-2}) = 0.001 (base, hatch door), 1.0 ng per sleeve
ND = not detected
(R) = right
(L) = left

Table 60. Amount of EPI Recovered from Isolator Gloves during Baseline 1 and 2

Area	Collection Point (Day and Session)							
	1.1	**1.2**	**2.1**	**2.2**	**3.1**	**3.2**	**4.1**	**4.2**
Baseline 1 (ng per glove)								
Isolator glove, right	975	733	562	404	1,506	274	239	409
Isolator glove, left	857	837	636	1,932	1,094	321	218	423
Baseline 2 (ng per glove)								
Isolator glove, right	ND	269	209	409	242	224	ND	ND
Isolator glove, left	ND	219	ND	1,591	210	1,830	ND	201

EPI LoD = 2.0 ng per glove ND = not detected

Table 61. Amount of EPI Recovered from Surfaces Outside the Isolator during Baseline 1 and 2

Area	Day and Sampling Point														
	1a	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**
Baseline 1 (ng cm^{-2})																
Floor	<0.005	0.11	ND	ND	0.09	0.09	0.10	<0.005	ND	ND	ND	ND	ND	ND	<0.005	ND
Tray in	0.06	0.07	0.06	0.19	0.06	0.06	ND	ND	0.10	0.07	0.07	0.08	0.06	ND	0.08	0.07
Tray out	<0.003	0.01	0.06	0.06	0.06	0.06	ND	ND	0.06	0.07	0.06	0.06	0.05	ND	0.07	<0.003
Baseline 2 (ng cm^{-2})																
Floor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<0.005	ND	ND	ND	ND
Tray in	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tray out	ND	ND	ND	ND	ND	ND	ND	ND	ND	<0.003	ND	<0.003	<0.003	ND	ND	ND

EPI LoD (ng cm^{-2}) = 0.002 (floor), 0.001 (tray) ND = not detected

Table 62. Amount of EPI Recovered from Support Gloves during Baseline 1 and 2

Area	Collection Point (Day and Session)							
	1.1	**1.2**	**2.1**	**2.2**	**3.1**	**3.2**	**4.1**	**4.2**
Baseline 1 (ng per glove)								
Support glove, right	3,600	926	1,319	205	12,823	1,148	225	165
Support glove, left	1,268	1,227	1,488	341	13,268	1,727	178	192
Baseline 2 (ng per glove)								
Support glove, right	ND	ND	ND	ND	ND	ND	ND	ND
Support glove, left	ND	ND	172	168	ND	ND	ND	ND

EPI LoD = 2.0 ng per glove ND = not detected

Table 63. Amount of EPI Recovered from Support Gloves during Intervention 1 and 2

Area	Collection Point (Day and Session)							
	1.1	**1.2**	**2.1**	**2.2**	**3.1**	**3.2**	**4.1**	**4.2**
Intervention 1 (ng per glove)								
Support glove, right	ND	ND	ND	ND	189	ND	ND	ND
Support glove, left	186	ND	ND	ND	1,046	ND	ND	ND
Intervention 2 (ng per glove)								
Support glove, right	ND	ND	172	ND	82.0	ND	ND	ND
Support glove, left	177	ND	ND	ND	ND	167	ND	ND

EPI LoD = 2.0 ng per glove ND = not detected

Table 64. Amount of EPI Recovered from Isolator Surfaces during Intervention 1 and 2

Area	Day and Sampling Point															
	1a	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**
Intervention 1 (ng cm^{-2}, or ng per sleeve)																
Base	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<0.002	ND	ND	ND
Door, outer (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, outer (L)	ND	ND	ND	ND	ND	ND	ND	ND	0.09	ND	0.11	ND	ND	ND	ND	ND
Door, inner (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<0.003
Door, inner (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve (R)	ND	41.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Intervention 2 (ng cm^{-2}, or ng per sleeve)																
Base	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, outer (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, outer (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, inner (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, inner (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

EPI LoD (ng cm^{-2}) = 0.001 (base, hatch door), 1.0 ng per sleeve
ND = not detected
(R) = right
(L) = left

Table 65. Amount of EPI Recovered from Isolator Gloves during Intervention 1 and 2

Area	Collection Point (Day and Session)							
	1.1	**1.2**	**2.1**	**2.2**	**3.1**	**3.2**	**4.1**	**4.2**
Intervention 1 (ng per glove)								
Isolator glove, right	ND	ND	ND	ND	ND	171	ND	ND
Isolator glove, left	ND	ND	ND	ND	ND	162	164	ND
Intervention 2 (ng per glove)								
Isolator glove, right	ND	ND	ND	ND	ND	ND	ND	ND
Isolator glove, left	ND	ND	ND	161	ND	ND	ND	ND

EPI LoD = 2.0 ng per glove ND = not detected

Table 66. Amount of EPI Recovered from Surfaces Outside the Isolator during Intervention 1 and 2

Area	Day and Sampling Point															
	1a	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**
Intervention 1 (ng cm^{-2})																
Floor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tray in	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tray out	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<0.003
Intervention 2 (ng cm^{-2})																
Floor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tray in	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tray out	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

EPI LoD (ng cm^{-2}) = 0.002 (floor), 0.001 (tray) ND = not detected

Table 67. Amount of EPI Recovered from Batches during Baseline 1 and 2

	Syringe									
Batch	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Baseline 1 (ng per syringe)										
batch 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 4	54.5	66.0	43.0	43.0	47.0	78.5	52.0	60.0	42.0	46.0
batch 8	<2.0	ND	<2.0	ND	<2.0	ND	<2.0	ND	41.5	<2.0
batch 12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 14	<2.0	59.5	61.5	46.5	52.5	52.0	57.5	53.5	ND	<2.0
batch 16	40.0	ND	<2.0	64.5	45.0	41.5	40.0	<2.0	<2.0	<2.0
batch 22	<2.0	<2.0	99.0	62.0	59.5	56.0	52.0	50.5	49.5	50.5
batch 24	ND	ND	ND	696	ND	ND	ND	ND	ND	ND
Baseline 2 (ng per syringe)										
batch 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 4	<2.0	<2.0	<2.0	<2.0	<2.0	ND	<2.0	ND	ND	<2.0
batch 8	ND	ND	ND	ND	<2.0	<2.0	<2.0	ND	ND	ND
batch 12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 22	1,807	53.0	46.0	44.0	49.0	41.0	41.0	40.5	40.5	92.0
batch 24	40.5	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0

EPI LoD = 1.0 ng per syringe ND = not detected

Table 68. Amount of EPI Recovered from Batches during Intervention 1 and 2

	Syringe									
Batch	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Intervention 1 (ng per syringe)										
batch 2	ND	ND	ND	ND	ND	ND	ND	<2.0	<2.0	ND
batch 4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Intervention 2 (ng per syringe)										
batch 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 22	ND	ND	ND	ND	<2.0	ND	ND	ND	ND	ND
batch 24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

EPI LoD = 1.0 ng per syringe ND = not detected

Appendix 8 – Methotrexate Surface Contamination Data

Table 69. Amount of MTX Recovered from Isolator Surfaces during Baseline 1 and 2

Area	Day and Sampling Point														
	1a	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**
Baseline 1 (ng cm^{-2}, or ng per sleeve)																
Base	ND	ND	ND	<0.04	ND	ND	ND	ND	0.33	0.26	0.18	0.19	0.13	<0.04	<0.04	0.09
Door, outer (R)	ND	ND	ND	<0.07	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.07	<0.07	ND
Door, outer (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<0.07	<0.07	ND
Door, inner (R)	ND	ND	<0.07	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.10	ND	0.22
Door, inner (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<0.07	<0.07	<0.07	ND
Sleeve (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<50.0	ND	ND	ND	ND	ND
Sleeve (L)	ND	ND	ND	<50.0	ND	ND	ND	ND	ND	<50.0	ND	ND	<50.0	<50.0	282	ND
Baseline 2 (ng cm^{-2}, or ng per sleeve)																
Base	ND	ND	ND	ND	ND	ND	ND	ND	0.60	ND	ND	ND	0.24	ND	0.27	0.24
Door, outer (R)	0.34	<0.07	<0.07	ND	ND	ND	ND	ND	ND	<0.07	ND	ND	0.11	<0.07	ND	0.15
Door, outer (L)	0.11	<0.07	ND	ND	ND	ND	ND	ND	ND	<0.07	ND	ND	0.14	0.16	0.19	0.24
Door, inner (R)	<0.07	ND	ND	ND	ND	ND	ND	8.04	ND	ND	ND	ND	0.20	0.19	<0.07	0.08
Door, inner (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	<0.07	ND	ND	0.15	<0.07	<0.07	<0.07
Sleeve (R)	216	ND	ND	ND	ND	ND	ND	ND	ND	<50.0	102	ND	198	ND	<50.0	ND
Sleeve (L)	191	<50.0	ND	ND	ND	ND	ND	ND	ND	<50.0	158	ND	<50.0	ND	211	716

MTX LoD (ng cm^{-2}) = 0.02 (base), 0.04 (hatch door), 25.0 ng per sleeve
ND = not detected
(R) = right
(L) = left

Table 70. Amount of MTX Recovered from Isolator Gloves during Baseline 1 and 2

Area	Collection Point (Day and Session)							
	1.1	**1.2**	**2.1**	**2.2**	**3.1**	**3.2**	**4.1**	**4.2**
Baseline 1 (ng per glove)								
Isolator glove, right	ND	748	ND	1,427	558	1,559	141	1,507
Isolator glove, left	ND	856	ND	797	715	133	153	298
Baseline 2 (ng per glove)								
Isolator glove, right	ND	ND	ND	ND	ND	ND	ND	748
Isolator glove, left	ND	ND	ND	ND	ND	ND	ND	750

MTX LoD = 50.0 ng per glove ND = not detected

Table 71. Amount of MTX Recovered from Surfaces Outside the Isolator during Baseline 1 and 2

Area	Day and Sampling Point															
	1a	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**
Baseline 1 (ng cm^{-2})																
Floor	ND	ND	ND	ND	ND	ND	<0.11	ND	ND	ND	<0.11	ND	ND	ND	ND	<0.11
Tray in	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<0.07	ND	0.19	<0.07	0.10
Tray out	ND	ND	ND	ND	ND	ND	ND	ND	0.14	0.21	<0.07	<0.07	ND	ND	<0.07	0.16
Baseline 2 (ng cm^{-2})																
Floor	11.3	ND	ND	<0.11	ND	ND	ND	ND	0.68	ND	ND	ND	ND	ND	<0.11	ND
Tray in	ND	ND	ND	ND	ND	ND	ND	ND	0.08	<0.07	ND	ND	ND	ND	0.07	ND
Tray out	ND	ND	ND	ND	ND	ND	ND	ND	ND	<0.07	ND	ND	ND	ND	<0.07	ND

MTX LoD (ng cm^{-2}) = 0.06 (floor), 0.04 (tray) ND = not detected

Table 72. Amount of MTX Recovered from Support Gloves during Baseline 1 and 2

Area	Collection Point (Day and Session)							
	1.1	**1.2**	**2.1**	**2.2**	**3.1**	**3.2**	**4.1**	**4.2**
Baseline 1 (ng per glove)								
Support glove, right	ND	ND	ND	ND	ND	ND	ND	ND
Support glove, left	ND	ND	ND	ND	ND	ND	ND	ND
Baseline 2 (ng per glove)								
Support glove, right	ND	ND	ND	ND	ND	ND	ND	ND
Support glove, left	ND	ND	ND	ND	ND	ND	ND	ND

MTX LoD = 50.0 ng per glove ND = not detected

Table 73. Amount of MTX Recovered from Support Gloves during Intervention 1 and 2

Area	Collection Point (Day and Session)							
	1.1	**1.2**	**2.1**	**2.2**	**3.1**	**3.2**	**4.1**	**4.2**
Intervention 1 (ng per glove)								
Support glove, right	ND	ND	ND	ND	ND	ND	ND	ND
Support glove, left	ND	ND	ND	ND	ND	ND	ND	ND
Intervention 2 (ng per glove)								
Support glove, right	ND	ND	ND	ND	ND	ND	ND	ND
Support glove, left	ND	ND	ND	ND	ND	ND	ND	ND

MTX LoD = 50.0 ng per glove ND = not detected

Table 74. Amount of MTX Recovered from Isolator Surfaces during Intervention 1 and 2

Area	Day and Sampling Point															
	1a	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**
Intervention 1 (ng cm^{-2}, or ng per sleeve)																
Base	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, outer (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, outer (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<0.07	ND	ND	ND
Door, inner (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.07	ND	ND	ND	ND	ND	ND
Door, inner (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Intervention 2 (ng cm^{-2}, or ng per sleeve)																
Base	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, outer (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, outer (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, inner (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, inner (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve (R)	ND	ND	ND	<50.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

MTX LoD (ng cm^{-2}) = 0.02 (base), 0.04 (hatch door), 25.0 ng per sleeve
ND = not detected
(R) = right
(L) = left

Table 75. Amount of MTX Recovered from Isolator Gloves during Intervention 1 and 2

Area	Collection Point (Day and Session)							
	1.1	**1.2**	**2.1**	**2.2**	**3.1**	**3.2**	**3.3**	**3.4**
Intervention 1 (ng per glove)								
Isolator glove, right	ND	ND	ND	ND	ND	ND	ND	ND
Isolator glove, left	ND	ND	ND	ND	ND	ND	ND	ND
Intervention 2 (ng per glove)								
Isolator glove, right	ND	ND	ND	ND	ND	ND	ND	ND
Isolator glove, left	ND	ND	ND	ND	ND	ND	ND	ND

MTX LoD = 50.0 ng per glove ND = not detected

Table 76. Amount of MTX Recovered from Surfaces Outside the Isolator during Intervention 1 and 2

Area	Day and Sampling Point															
	1a	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**
Intervention 1 (ng cm^{-2})																
Floor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tray in	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tray out	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.14	ND	ND	ND
Intervention 2 (ng cm^{-2})																
Floor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tray in	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tray out	ND	0.39	0.35	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

MTX LoD (ng cm^{-2}) = 0.06 (floor), 0.04 (tray) ND = not detected

Table 77. Amount of MTX Recovered from Batches during Baseline 1 and 2

	Syringe									
Batch	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Baseline 1 (ng per syringe)										
batch 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 4	ND	ND	ND	ND	205	ND	ND	ND	ND	ND
batch 8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 12	ND	ND	73.0	ND	ND	ND	ND	ND	ND	ND
batch 14	ND	ND	<50.0	ND	ND	ND	ND	ND	ND	ND
batch 16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 22	ND	ND	ND	ND	ND	<50.0	ND	ND	ND	ND
batch 24	259	ND	ND	ND	ND	ND	ND	ND	ND	ND
Baseline 2 (ng per syringe)										
batch 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 16	ND	ND	ND	ND	ND	<50.0	<50.0	ND	<50.0	ND
batch 22	ND	ND	ND	ND	ND	ND	ND	<50.0	ND	ND
batch 24	ND	ND	ND	ND	ND	ND	<50.0	ND	<50.0	<50.0

MTX LoD = 25.0 ng per syringe ND = not detected

Table 78. Amount of MTX Recovered from Batches during Intervention 1 and 2

	Syringe									
Batch	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Intervention 1 (ng per syringe)										
batch 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Intervention 2 (ng per syringe)										
batch 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 24	ND	ND	81.5	ND	ND	ND	ND	ND	ND	ND

MTX LoD = 25.0 ng per syringe ND = not detected

Appendix 9 – Cyclophosphamide Surface Contamination Data

Table 79. Amount of CP Recovered from Isolator Surfaces during Baseline 1 and 2

Area	Day and Sampling Point															
	1a	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**
Baseline 1 (ng cm^{-2}, or µg per sleeve)																
Base	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, outer (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	117	ND	ND	ND	ND	ND
Door, outer (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	135	ND	ND	ND	ND
Door, inner (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, inner (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Baseline 2 (ng cm^{-2}, or µg per sleeve)																
Base	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, outer (R)	ND	ND	426	ND	ND	ND	ND	ND	ND	269	174	ND	ND	ND	ND	ND
Door, outer (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	190	215	393	ND	ND	ND	ND
Door, inner (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	302	368	982	ND	ND	ND	ND
Door, inner (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	237	221	2,034	ND	ND	ND	ND
Sleeve (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	304	264	ND	ND	ND	ND	ND
Sleeve (L)	ND	ND	134	ND	ND	ND	ND	ND	ND	278	266	ND	ND	ND	ND	ND

CP LoD (ng cm^{-2}) = 9.50 (base), 17.5 (hatch door), 12.5 µg per sleeve
ND = not detected
(R) = right
(L) = left

Table 80. Amount of CP Recovered from Isolator Gloves during Baseline 1 and 2

Area	Collection Point (Day and Session)							
	1.1	**1.2**	**2.1**	**2.2**	**3.1**	**3.2**	**4.1**	**4.2**
Baseline 1 (µg per glove)								
Isolator glove, right	ND	ND	ND	ND	ND	ND	ND	ND
Isolator glove, left	ND	ND	ND	ND	ND	ND	ND	ND
Baseline 2 (µg per glove)								
Isolator glove, right	ND	ND	ND	192	ND	ND	ND	ND
Isolator glove, left	ND	ND	ND	ND	ND	ND	ND	ND

CP LoD = 25.0 µg per glove ND = not detected

Table 81. Amount of CP Recovered from Surfaces Outside the Isolator during Baseline 1 and 2

Area	Day and Sampling Point															
	1a	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**
Baseline 1 (ng cm^{-2})																
Floor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tray in	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tray out	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Baseline 2 (ng cm^{-2})																
Floor	ND	ND	ND	ND	ND	ND	ND	ND	ND	128	113	119	ND	ND	ND	ND
Tray in	ND	383	72.6	384	ND	ND	ND	ND	ND	155	137	ND	ND	ND	ND	ND
Tray out	ND	ND	131	118	ND	ND	ND	ND	ND	137	74.7	ND	ND	ND	ND	ND

CP LoD (ng cm^{-2}) = 28.3 (floor), 18.0 (tray) ND = not detected

Table 82. Amount of CP Recovered from Support Gloves during Baseline 1 and 2

Area	Collection Point (Day and Session)							
	1.1	**1.2**	**2.1**	**2.2**	**3.1**	**3.2**	**4.1**	**4.2**
Baseline 1 (µg per glove)								
Support glove, right	756	410	1,063	1,125	1,216	1,186	ND	ND
Support glove, left	754	623	108	1,339	1,262	1,356	884	ND
Baseline 2 (µg per glove)								
Support glove, right	578	823	946	635	913	195	97.6	1,111
Support glove, left	946	893	843	874	678	944	612	866

CP LoD = 25.0 µg per glove ND = not detected

Table 83. Amount of CP Recovered from Support Gloves during Intervention 1 and 2

Area	Collection Point (Day and Session)							
	1.1	**1.2**	**2.1**	**2.2**	**3.1**	**3.2**	**4.1**	**4.2**
Intervention 1 (µg per glove)								
Support glove, right	96.0	315	724	1,146	694	453	456	385
Support glove, left	693	199	815	873	596	488	584	470
Intervention 2 (µg per glove)								
Support glove, right	334	395	812	728	407	46.0	865	788
Support glove, left	1,251	1,086	1,342	1,099	1,076	1,027	1,431	1,476

CP LoD = 25.0 µg per glove

Table 84. Amount of CP Recovered from Isolator Surfaces during Intervention 1 and 2

Area	Day and Sampling Point															
	1a	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**
Intervention 1 (ng cm^{-2}, or ng per sleeve)																
Base	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, outer(R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, outer (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, inner (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, inner (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Intervention 2 (ng cm^{-2}, or ng per sleeve)																
Base	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, outer (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, outer (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, inner (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Door, inner (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve (R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve (L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

CP LoD (ng cm^{-2}) = 9.50 (base), 17.5 (hatch door), 12.5 µg per sleeve
ND = not detected
(R) = right
(L) = left

Table 85. Amount of CP Recovered from Isolator Gloves during Intervention 1 and 2

Area	Collection Point (Day and Session)							
	1.1	**1.2**	**2.1**	**2.2**	**3.1**	**3.2**	**4.1**	**4.2**
Intervention 1 (µg per glove)								
Isolator glove, right	ND	ND	ND	ND	ND	ND	ND	ND
Isolator glove, left	ND	ND	ND	ND	ND	ND	ND	ND
Intervention 2 (µg per glove)								
Isolator glove, right	ND	ND	ND	ND	138	ND	ND	ND
Isolator glove, left	ND	ND	ND	192	ND	ND	ND	ND

CP LoD = 25.0 µg per glove ND = not detected

Table 86. Amount of CP Recovered from Surfaces Outside the Isolator during Intervention 1 and 2

Area	Day and Sampling Point															
	1a	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**
Intervention 1 (ng cm^{-2})																
Floor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tray in	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	64.7	65.5	214	ND
Tray out	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	66.1	183	ND
Intervention 2 (ng cm^{-2})																
Floor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tray in	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	66.1	91.2	ND	68.2	66.8	ND
Tray out	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

CP LoD (ng cm^{-2}) = 28.3 (floor), 18.0 (tray) ND = not detected

Table 87. Amount of CP Recovered from Batches during Baseline 1 and 2

	Syringe									
Batch	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Baseline 1 (µg per syringe)										
batch 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 8	ND	ND	ND	35.5	41.5	ND	ND	ND	ND	ND
batch 12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Baseline 2 (µg per syringe)										
batch 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 22	ND	ND	ND	ND	ND	57.5	59.5	74.0	75.0	ND
batch 24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

CP LoD = 12.5 µg per syringe ND = not detected

Table 88. Amount of CP Recovered from Batches during Intervention 1 and 2

	Syringe									
Batch	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Intervention 1 (µg per syringe)										
batch 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Intervention 2 (µg per syringe)										
batch 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
batch 24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

CP LoD = 12.5 µg per syringe ND = not detected